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# Sugarcane
## Technology and Research

*Edited by Alexandre Bosco de Oliveira*

# SUGARCANE - TECHNOLOGY AND RESEARCH

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# Meet the editor

Alexandre Bosco de Oliveira has a BS degree in Agronomic Engineering (2006) and Secondary Biology Education (2009). He obtained his MS (2008) and PhD (2010) degrees in Agronomy/Crop Science at the Federal University of Ceará (UFC), Brazil, and has worked as a visiting researcher at the University of Florida (2017), USA. His academic background involves research activities with seedling production, plant propagation, germination ecophysiology, and physiology of water and salt stress. In 2012, he started his current position as a professor of Agriculture at UFC. Since then, he has been the head of the Crop Physiology Laboratory and coordinates the Ecophysiology of Semiarid Agroecosystems Research Group, advising undergraduate and graduate students. He published 4 books, 20 chapters in books, and 51 articles in specialized scientific journals. He also contributes as a reviewer and an associate editor of relevant scientific journals worldwide.

# Contents

# Preface

Renewable energy has been considered one of the most relevant tools to reduce emissions of carbon dioxide and avoid other environmental issues. In this context, the constant pursuit to achieve sustainability has led farmers and scientists to go one step further through agriculture, using bioenergy crops. When it comes to production of these types of specialized species, sugarcane (*Saccharum officinarum L.*) is considered a successful and efficient model, especially in tropical and subtropical regions. Moreover, its industry represents one of the most important economic activities worldwide, providing about 80% of the sugar consumed in the planet and reducing emissions of carbon dioxide by hundreds of million tons in the last few years.

Sugarcane is grown commercially in the tropics and subtropics, and is known to be one of the oldest cultivated plants in the world. Nonetheless, facing the increasing population, and consequently, higher demand for sugar and biofuels, the demand for sugarcane and its by-products is set to increase in the next years. Thus, sugarcane research to improve sustainable production worldwide is the vital task of scientific community to address the increasing demands and needs for their products. In the last few decades, the results of wide-scale research have been applied, field mechanization has been improved, technical and agricultural methods have been developed, research and training extended and new methods established worldwide.

This book intends, therefore, to cover some of the most important agricultural results of this progress, describing and discussing challenges that came up with the advent of next generation technologies. Hence, this volume presents a collection of 14 chapters involving theoretical and practical research work carried out by experienced researchers working with this crop. Many authors are recognized as leading experts in their field and provide unique perspectives as a result of their many years of experience.

The literature presented here is a comprehensive overview of current concepts related to the production of sugarcane and its industrial applications. In this context, the chapters have been classified into five sections highlighting fundamental insights associated with this crop's current research and technology. The sections consist of the following topics: "Sustainability and Economics", "Bionergy and Carbon Sequestration", "Propagation and Biotechnology", "Fertilization and Harvest", and "Industrial Applications".

This is a definitely a valuable reference book on sugarcane production, agronomy, and end-use qualities, particularly for those who work in research organizations and higher academic institutions. It will be of great interest, especially, to graduates, postgraduates and researchers who work with agriculture, biotechnology, chemistry and related subjects. Moreover, it provides a reasonable resource for readers interested in a quick review of trending topics about this crop.

As the editor of this book, I am grateful to all the authors who have written their chapters meticulously and contributed their valuable work. I also would like to thank to the editorial staff of the publisher IntechOpen, and their team, for all the kind support provided throughout the whole editorship process, enabling to produce this book on time and in a great manner. I express my special thanks to my mother Francisca, my wife Maria, and my kids Matheus and Giovana, for inspiring me and being my pillars of strength. Last but not the least, my deepest gratitude is for my Lord and Savior, Jesus Christ, who takes care of me and gives me health to make my dreams come true. "I will give thanks to you, LORD, with all my heart; I will tell of all your wonderful deeds." Ps. 9:1

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Section 1

# Sustainability and Economics

Chapter 1

# Multi-Analytical Interactions in Support of Sugarcane Agroecosystems Sustainability in Tropical Soils

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**Abstract**

The risks of sugarcane management on soil microbes and their relationships with soil physicochemical factors and biogeochemical processes have not been described from an integrated perspective for different agronomic practices. Here, we provide a platform for multi-analytical interactions between ecologists analyzing the soil microbes at multiple ecological levels and geoscientists measuring the release of greenhouse gases and the physicochemical soil factors including labile fractions from soil organic matter in tropical sugarcane management systems. We compile the benefits and risks of nutrient management and soil amendments as well as of crop residue and harvest management in sugarcane soils on belowground microbial life and biogeochemical processes mediated by soil microbial communities, and we demonstrate that the massive planting of the crop brings environmental risks that include a potential impact on tropical soil ecosystem sustainability. We emphasize that soil management and harvest management are critical for supporting the sustainable development of biofuel production in tropical areas.

**Keywords:** soil microbes, biogeochemical processes, greenhouse gases, soil management, harvest management, ecosystem sustainability

## 1. Introduction

Although sugarcane (*Saccharum* spp.) has been traditionally cultivated for sugar production, it has emerged in the past few decades as one of the best crops for biofuel production [1]. Currently, world sugarcane production is close to 1.6 billion tons annually and is concentrated in the tropical regions, particularly in the developing nations in Latin America, Africa, and Asia [2] (**Figure 1**). Brazil is the world's largest sugarcane producer, followed by India, China, Pakistan, Thailand, and Mexico. As a result of the increased economic importance of sugarcane, the requirements for large-scale production in an environmentally sustainable manner have also increased. However, massive planting of the crop brings environmental risks that include a potential impact on tropical soil ecosystem sustainability (**Figure 1**), which is still an open question for soil microbes and microbial-mediated processes that lead to greenhouse gas (GHG) emissions.

Soil functions are effective only as long as the capacity for the interactions between the physical, chemical, and biological processes is preserved. The increased need for fertilizers due to the expansion of sugarcane production is a threat to the ability of the soil to maintain its potential for self-regulation in the long term, i.e., its sustainability [3]. Soil management practices used in sugarcane agriculture require synthetic mineral fertilizers (nitrogen/phosphorus/potassium—NPK) [4] and full recycling of waste products from the ethanol production to sugarcane fields in the form of organic fertilizer [5]. Sugarcane vinasse is a by-product of the

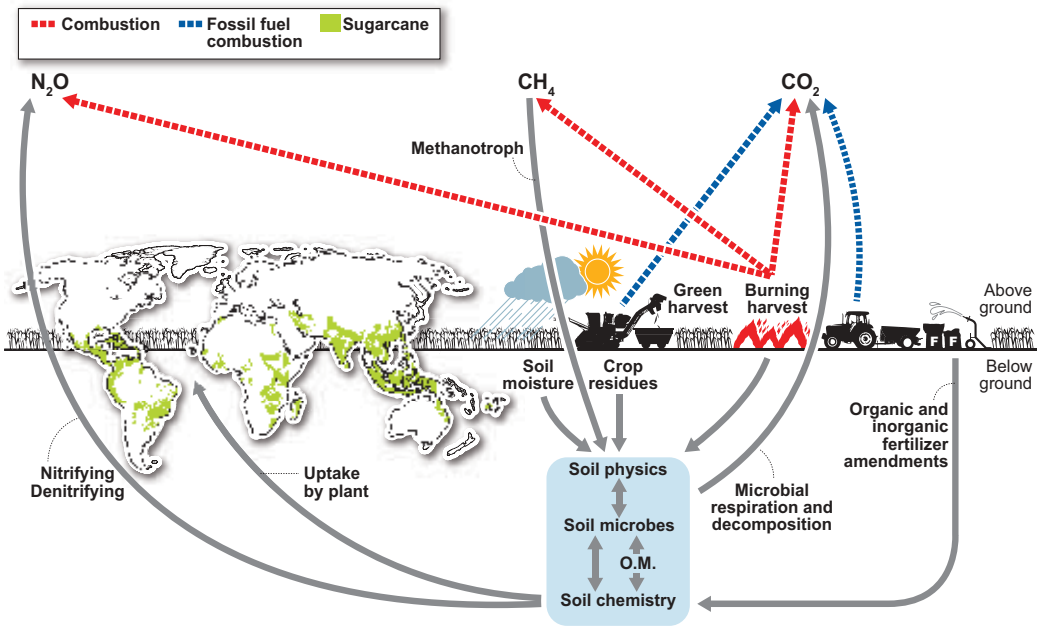


**Figure 1.** Infographic of the belowground-atmospheric potential impacts of large-scale sugarcane production from a soil ecological and integrated perspective. The map shows sugarcane production in the world. Gas emissions from combustion are shown from burning harvest. Carbon dioxide ($CO_2$) emissions are shown from fossil fuel combustion aboveground.

sugar-ethanol industry, also known as stillage [6]. Its chemical composition varies depending on the mill plant used for the production of ethanol and the distillation process [7]. In general, sugarcane vinasse is composed of water (93%) and organic solids and minerals (7%) [7]. It has high levels of organic matter but is low in N and P. The main non-water component of sugarcane vinasse is organic matter that exists in the form of organic acids and cations such as K, calcium (Ca), and magnesium (Mg) [7]. Since the 1960s, vinasse has been used as a liquid fertilizer in the sugarcane fields of Brazil to solve the ecological problem of its disposal within the environment. Studies from the late 1980s have recommended the use of N fertilizer in combination with vinasse in sugarcane fields [8], and a more recent study has recommended the use of N fertilizer with straw retention [9].

The inorganic and organic fertilizer amendments, primarily used to increase nutrient availability to plants, and the management of sugarcane harvest residue are likely to affect the physical [10, 11], chemical [10, 12–14], and microbiological [13–21] attributes of sugarcane soils as well as the GHG emissions from sugarcane areas [19, 22–26]. Soil microbes comprise a major fraction of the total living soil biomass [27]. Many of the abovementioned studies have highlighted that numerous microbial groups are highly correlated with specific soil factors. The studies reported differences in the soil microbial community related to management practices for sugarcane due to the effects of soil factors. Despite increased attention to the soil microbial community and its relationship with soil characteristics in sugarcane-cultivated areas, little progress has been made in elucidating the implications of the agricultural practices on the functional roles of this community in tropical sugarcane agriculture [16].

With this in mind, this chapter was aimed at examining the available data on the subject as a contribution to update the knowledge on the benefits and risks of nutrient management and soil amendments as well as of crop residue and harvest management in sugarcane soils on belowground microbial life, soil physical and chemical factors, and biogeochemical processes mediated by soil microbial communities. We summarize, in this chapter, the impacts of these management practices on soil microbes at multiple ecological levels, on soil physicochemical attributes including labile fractions from soil organic matter and on GHG emissions (mainly nitrous oxide due to nitrogen losses in sugarcane production systems). Based on multi-analytical interactions, we emphasize that soil management and harvest management are critical for supporting the sustainable development of biofuel production.

## 2. Nutrient management and soil amendments

Sugarcane is a semi-perennial crop replanted after 3–7 ratoon cycles, depending at least in part on the soil fertility and crop variety [5]. After a relatively long time receiving fertilizers and recycling crop residue on an annual basis, the soil ecosystem sustainability and multifunctionality can become compromised in most production areas [28]. The impacts of these management practices, and inorganic and organic fertilizer amendments on soil microbes and GHG emissions, as well as on soil physicochemical factors including labile fractions from soil organic matter in sugarcane fields worldwide are addressed below based on a soil multi-analytic perspective.

### 2.1. Application of mineral fertilizers

Annually, sugarcane production fields are amended with inorganic sources of N, P, and K as well as other, more sporadic, amendments, such as Ca, Mg, sulfur (S), and micronutrients. However, the macronutrients, such as P, K, Ca, Mg, and S, are also fundamental for the development of sugarcane and when used in association, they could reflect increases of productivities.

Urea is considered the most widely used N fertilizer in sugarcane fields, followed by ammonium sulfate and ammonium nitrate [29]. However, more than 25% of the N applied in the form of urea to surface soil during the sugarcane ratoon cycles can be volatilized to ammonia [29]. Consequently, urea is applied only during the sugarcane vegetative stage in Brazil. The use of liquid urea with crop residue blankets has been reported to avert N volatilization in Australia [30]. Both urease and nitrifier inhibitors can alternatively be used to reduce N losses as ammonia [31].

Nitrification, i.e., the biological oxidation of ammonia into nitrite, followed by the oxidation of nitrite into nitrate can produce nitrous oxide ($N_2O$) as a by-product. Soares et al. [19] reported reduced $N_2O$ emissions from a sugarcane field in Brazil after DMPP (3,4-dimethylpyrazole phosphate)-coated urea applications (**Table 1**), with fewer effects on the microbial community diversity and composition in comparison with treatments using urea or calcium nitrate. However, Wang et al. [32] did not find similar results for well-drained soil in Australia (**Table 1**), even after applying three times more DMPP-coated urea than that used on the Brazilian soil. These results may be at least in part due to the expected differences in soil microbial communities between the soil types and geographical regions.

Archaea and bacteria are key drivers of N in the redox process of denitrification of nitrate to form $N_2O$ in the soil [20]. Soares et al. [19] showed that $N_2O$ emissions in sugarcane soils were significantly correlated with bacterial *amo*A genes but not with denitrification-related genes (*nir*K, *nir*S, and *nos*Z), suggesting that ammonia-oxidizing bacteria via nitrification are the main contributors to emissions of $N_2O$ when urea is used as a fertilizer. In turn, Fracetto et al. [33] showed an increase in denitrifying gene abundance (*nir*S, *nir*K, *nor*B, and *nos*Z) after ammonium nitrate application to the soil, with $N_2O$ emissions associated with *nor*B gene abundance. However, denitrification may contribute to much of the $N_2O$ emissions from sugarcane cultivation systems [19, 34], and denitrification is at least in part associated with soil moisture content [35]. In soils with 75% water-filled pore space (WFPS), denitrification has been shown to be the most important process in $N_2O$ emissions, while nitrification has been shown to be the most important process in soils with 60% WFPS [35]. Denitrification is a respiratory process that regularly occurs in the absence of $O_2$, in which $NO_3^-$ is used as an electron acceptor. However, although large denitrification rates are associated with low concentrations of $O_2$, aerobic denitrification has also been demonstrated for some bacteria [34].

The N fertilizer dose has been associated with changes in microbial communities [13, 17] and in abundance of functional genes associated with nitrification and denitrification in the sugarcane soil and rhizosphere [36]. Although fungal species richness in the sugarcane soil and rhizosphere has not shown variation to N fertilizer applied to the soil at different

rates, changes in *Ascomycota* and *Basidiomycota* abundance were detected in these soils, with *Basidiomycota* abundance negatively affected by increasing N dose [17]. Gumiere et al. [18] evaluated the diversity and abundance of fungal communities in soils used for the cultivation of sugarcane and demonstrated that the distribution of fungal species abundance fits better a neutral model that assumes biogeographical patterns than models that assume environmental filtering. Recently, fungi have being presented as contributors to the $N_2O$ released from soils, and pH was the parameter that explained the majority of this share [37]. Nitrous oxide production was confirmed *in vitro* as a common trait of fungi [38]. Considering the relevance of pH to the $N_2O$ emissions attributed to fungi, this subject needs to be covered to understand the processes that result in the release of gas in sugarcane soils, since the crop grows predominantly in acidic soils (**Table 1**). In addition, since N application changes the fungal community, it may also change the balance of $N_2O$ produced by this group of soil microorganisms.

| Reference | N dose (kg ha$^{-1}$) | Crop stage | Straw blanket | Soil type | Redox status | N source | Annual N-$N_2O$ emission (g ha$^{-1}$) | Soil pH | Soil OM (%) | Sampling events | Time covered (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Soares et al. [19] | 0 | 3rd ratoon | Removed | Oxisol | Well-drained | – | 286 | 5.1 | 2.3 | 41 | 278 |
| | 120 | | | | | Urea | 2301 | | | | |
| | 120 | | | | | Urea + DCD | 531 | | | | |
| | 120 | | | | | Urea + DCD-R | 350 | | | | |
| | 120 | | | | | Urea + DMPP | 2165 | | | | |
| | 120 | | | | | Urea + DMPP-R | 410 | | | | |
| | 120 | | | | | PSCU | 353 | | | | |
| | 120 | | | | | $Ca(NO_3)_2$ | 329 | | | | |
| Wang et al. [32] | 0 | 5th ratoon | 9.4 t ha$^{-1}$ | Lixisol | Well-drained | – | 1700 | 4.8 | 2.8[d] | 38 | 328 |
| | 80 | | | | | Urea | 2600 | | | | |
| | 150 | | | | | Urea | 3600 | | | | |
| | 80 | | | | | PCU | 3952[a] | | | | |
| | 80 | | | | | DMPP | 2300[b] | | | | |
| | 80 | | Removed | | | Urea | 1976[c] | | | | |
| | 0 | 1st ratoon | Burnt (2.9 t ha$^{-1}$ remained) | Gleysol | Flood-plain | – | 12,200 | 4.9 | 16.9[d] | 38 | 343 |
| | 80 | | | | | Urea | 23,200 | | | | |
| | 160 | | | | | Urea | 28,200 | | | | |
| | 80 | | | | | PCU | 16,100 | | | | |
| | 80 | | | | | DMPP | 20,700 | | | | |
| | 80 | | removed | | | Urea | 16,000 | | | | |

| Reference | N dose (kg ha$^{-1}$) | Crop stage | Straw blanket | Soil type | Redox status | N source | Annual N-$N_2O$ emission (g ha$^{-1}$) | Soil pH | Soil OM (%) | Sampling events | Time covered (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Carmo et al. [23] | 0 | 1st ratoon | Removed | Oxisol | Well-drained | – | 107 | 4.5 | 2.2 | 21 | 335 |
| | 120 | | Removed | | | $NH_4NO_3$ | 2091 | | | | |
| | 120 | | 7 t ha$^{-1}$ | | | $NH_4NO_3$ | 3286 | | | | |
| | 120 | | 14 t ha$^{-1}$ | | | $NH_4NO_3$ | 3019 | | | | |
| | 120 | | 21 t ha$^{-1}$ | | | $NH_4NO_3$ | 4170 | | | | |
| | 142 | | Removed | | | $NH_4NO_3$ + Vin. | 3024 | | | | |
| | 142 | | 7 t ha$^{-1}$ | | | $NH_4NO_3$ + Vin. | 5869 | | | | |
| | 142 | | 14 t ha$^{-1}$ | | | $NH_4NO_3$ + Vin. | 7034 | | | | |
| | 142 | | 21 t ha$^{-1}$ | | | $NH_4NO_3$ + Vin. | 7464 | | | | |
| | 0 | Plant cane | – | Lixisol | Well-drained | – | 577 | 4.5 | 2.2 | 20 | 314 |
| | 60 | | – | | | Urea | 1377 | | | | |
| | 60 | | – | | | Urea + Vin. | 2212 | | | | |
| | 85 | | – | | | Urea + Vin. | 3261 | | | | |
| | 85 | | – | | | Urea + Vin. + FC | 3566 | | | | |
| Pitombo et al. [26] | 0 | 1st ratoon | Removed | Oxisol | Well-drained | – | 1605 | 5.1 | 2.3 | 48 | 274 |
| | 100 | | | | | $NH_4NO_3$ | 1811 | | | | |
| | 161 | | | | | $NH_4NO_3$ + Vin. | 3763 | | | | |
| | 61 | | | | | Vin. | 2583 | | | | |
| | 37 | | | | | Concentrated Vin. | 2106 | | | | |
| | 0 | | 10 t ha$^{-1}$ | | | ___ | 1810 | | | | |
| | 100 | | | | | $NH_4NO_3$ | 2870 | | | | |
| | 161 | | | | | $NH_4NO_3$ + Vin. | 5699 | | | | |
| | 61 | | | | | Vin. | 3490 | | | | |
| | 37 | | | | | Concentrated Vin. | 2500 | | | | |
| Pitombo et al. [20] | 100 | 2nd ratoon | 0 t ha$^{-1}$ | Oxisol | Well-drained | $NH_4NO_3$ | 5237 | 5.2 | 2.8 | 37 | 246 |
| | 100 | | 5.6 t ha$^{-1}$ | | | $NH_4NO_3$ | 4548 | | | | |
| | 100 | | 8.5 t ha$^{-1}$ | | | $NH_4NO_3$ | 3204 | | | | |
| | 100 | | 11.3 t ha$^{-1}$ | | | $NH_4NO_3$ | 3347 | | | | |

| Reference | N dose (kg ha$^{-1}$) | Crop stage | Straw blanket | Soil type | Redox status | N source | Annual N-$N_2O$ emission (g ha$^{-1}$) | Soil pH | Soil OM (%) | Sampling events | Time covered (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Allen et al. [30] | 0 | 3rd and 4th ratoon | Kept | Hydrosol | Floodplain | – | 2860 | ~5 | 5.2[d] | 30 | ~365 |
| | 2 × 50 | | | | | Liquid urea | 3860 | | | | |
| | 100 | | | | | Liquid urea | 3930 | | | | |
| | 2 × 100 | | | | | Liquid urea | 5810 | | | | |
| | 200 | | | | | Liquid urea | 9560 | | | | |
| Paredes et al. [39] | 0 | 2nd ratoon | Kept | Oxisol | Well-drained | – | 3920 | 5.4 | 2.6[d] | 69 | 211 |
| | 100 | | | | | $(NH_4)_2SO_4$ | Not presented | | | | |
| | 118 | | | | | $(NH_4)_2SO_4$ + Vin. | | | | | |
| | 118 | | | | | $(NH_4)_2SO_4$ + Vin. | | | | | |
| | 18 | | | | | Vin. | | | | | |
| | 18 | | | | | Vin. | | | | | |

DCD, dicyandiamide; DMPP, dimethylpyrazole phosphate; PSCU, polymer sulfur coated urea; PCU, polymer coated urea; Vin., vinasse; FC, filter cake.

[a]Calculated based on data available at Results section (52% higher than treatment with 80 kg urea).

[b]Estimated from the plot.

[c]Calculated based on data available at Results section (24% lower than treatment with 80 kg urea).

[d]Obtained based on TOC*1.724.

**Table 1.** Annual nitrous oxide ($N_2O$) emissions from sugarcane production fields after nitrogen fertilizers applications to tropical soils with contrasting characteristics.

Sugarcane fields are widely distributed around the globe in tropical regions, and the crop grows both in deep well-drained soils and in floodplains (**Figure 1** and **Table 1**). This contrast limits the conclusion about which processes predominate in sugarcane fields. While the emissions can reach more than 20 kg ha$^{-1}$ N-$N_2O$ y$^{-1}$ in the floodplains, the amount drops to approximately 2.4 kg ha$^{-1}$ N-$N_2O$ y$^{-1}$, on average, in well-drained soils. When analyzing the effect of water saturation on $N_2O$ fluxes, Denmead et al. [40] verified that at 70% of WFPS, the $N_2O$ fluxes reach their highest values in the field and that this result would be due to the sum of $N_2O$ produced by both nitrification and denitrification processes. However, this hypothesis still needs to be addressed in a variety of soils to improve the understanding of the processes that result in $N_2O$ release in sugarcane soils.

Concerning the GHG emissions in sugarcane soils amended with mineral fertilizers, the emissions based on ammonium nitrate sources can vary from 1811 g ha$^{-1}$ to 5237 g ha$^{-1}$ [20, 26], and from 0.85 to 1.68% when urea is applied to the Brazilian tropical soils [19, 23, 32]. In Australia, the amount of $N_2O$ released and, consequently, the fertilizer emission factor vary broadly depending on the soil redox status and N dose applied (**Table 1**). The emission factor

for flooded areas has reached values higher than 20% [32, 40]; for well-drained areas, it has reached up to 1% for the standard fertilizer doses, but it increases for higher N doses [30].

The carbon dioxide ($CO_2$) and methane ($CH_4$) emissions in sugarcane soils are also directly related to the N fertilizer dose [23] and its effects on the metabolizable nutrient availability [22] and the soil microbial community [13]. Urea may be metabolized by *Nitrospira*, resulting in ammonia and $CO_2$ [41]. For instance, urea applied in pure form or as part of other organic amendments is hydrolyzed and results in $CO_2$. Urease is produced by a broad range of soil organisms—from bacteria to plants [42]. There are also possible indirect effects. The N fertilizers in agriculture also affect the soil capacity to consume $CH_4$ [43]. The oxidation of $NH_4^+$ and $CH_4$ are homologous functions, and they can be mediated by the same enzyme in methane-oxidizing bacteria and ammonia-oxidizing bacteria [44, 45]. This implies that $NH_4^+$ can inhibit the oxidation of $CH_4$ by competing for active sites [43, 45]. The specificity of a bacterial group in relation to another can cause the collapse of competition between groups either because of lack of energy or source of C, since the accumulation of toxic species of N follows the evolution of the oxidation of $NH_4^+$, which results in low consumption of $CH_4$ and greater availability of N for nitrification, denitrification, and formation of $N_2O$.

### 2.2. Use of organic fertilizers

As an alternative to mineral fertilization in sugarcane production fields, waste products from ethanol production (vinasse and filter cake), sewage sludge, green manures, inoculants of atmospheric N-fixing bacteria and phytohormones are commonly applied to the soil in the form of organic fertilizer to promote plant growth [46]. These organic fertilizers represent an important contribution of the N, P, K, and organic matter, mainly soil labile organic fractions, such as dissolved organic C and N, and others C-light organic fractions [47–49], in the sugarcane agroindustry [25]. Soil labile organic C can be defined as the soil organic matter fraction that sustains the soil food web and therefore directly influences nutrient cycles and many biologically related soil properties [50].

The filter cake, a solid organic residue of the sugarcane processing in the mill that is rich in P, is used mainly in cane-plants, at 10–30 t $ha^{-1}$ when applied in the furrow and, 80–100 t $ha^{-1}$ when applied in the total area, in pre-planting, replacing the phosphate fertilization partially or totally, depending on the dose of $P_2O_5$ recommended. The vinasse is mainly used in sugarcane, supplying all the $K_2O$ and part of the N, being very poor in P. Vinasse, depending on its chemical composition and soil fertility, is applied in the range of 60–120 $m^3$ $ha^{-1}$ by tank vehicles or 150–250 $m^3$ $ha^{-1}$ by irrigation-sprinkler [51].

Although organic fertilizers are used to increase sugarcane productivity through nutrient availability to plants, they can also affect soil microbial community and physicochemical soil factors [7, 14, 20, 52], and key biogeochemical processes associated with GHG emissions, such as decomposition, respiration, nitrification and denitrification [23, 25, 53]. Moreover, the use of organic residues has resulted in the increase of C and N labile organic forms [47–49], which has been used as soil quality indicator due to rapid alteration according to soil practice management [54]. It is generally assumed that plant litter and humus are the two most important

sources of dissolved organic matter in soils, and its release into solution occurs through physicochemical decomposition and leaching from litter and formation of humic substances [55].

Omori et al. [52] reported increases in bacterial diversity after vinasse application to the soil and revealed that this by-product of the sugar-ethanol industry promotes the participation of soil microbial community members in N and Fe cycling. The authors showed that *Acidobacteria* Gp3 and Gp4 were most abundant in the vinasse-amended soil. In addition, bacterial community members belonging to *Actinomycetales* were more diverse in vinasse-amended soil than in soils without vinasse. Navarrete et al. [14] reported effects of combined applications of vinasse and N fertilizer to the soil on bacterial communities in sugarcane soils. *Acidobacteria*, *Actinobacteria*, and *Verrucomicrobia* were the bacterial phyla most affected in these soils. The authors identified increases in $CO_2$ and $N_2O$ emissions shortly after the addition of both vinasse and N fertilizer to the soils, thus increasing the microbial-N biomass, decreasing the microbial-C biomass and altering the soil chemical factors that were correlated with the microbial biomass. Regarding the soil chemical factors, the K and S were negatively correlated with microbial biomass and the soil pH was positively correlated with microbial-C biomass. The long-term organic inputs has evidenced clear trend of increasing microbial-C biomass when compared with conventional practice management [47, 56]. In turn, Dias [53] reported that vinasse can increase the abundance of nitrous oxide reductase (*nos*Z) gene but not the copy number of both nitrite reductase (*nir*K) and methyl coenzyme-M reductase (*mcr*A) genes in sugarcane soils.

While vinasse is broadcast on the soil during the vegetative stage and on the ratoons, filter cake is typically used only during the vegetative sugarcane stage with mineral fertilizer added in the furrows (**Table 1**). Using a molecular approach based on 16S rRNA gene sequencing, Omori [57] revealed *Actinobacteria* as the predominant phylum in the bacterial community related to the degradation of plant biomass and the production of antimicrobials in sugarcane soil containing filter cake semi-composting, which is possibly related to the high amount of lignocellulosic material available in the filter cake. The authors also reported *Firmicutes* and *Proteobacteria* in the soil at different stages of the composting process. In turn, Hernández et al. [58] used a culture-dependent approach and showed that filter cake application to the sugarcane soil increases colonies of phosphate-solubilizing microorganisms, total bacteria, and fungi. In addition, Tellechea et al. [59] showed higher microbial activity in sugarcane soil with filter cake application based on traditional methods of $CO_2$ determination. These results are an important indicative that the microorganisms present in the filter cake are able to increase available P in the soil solution and then to improve its absorption by plants, which can be highlighted in the tropical soil condition, such as Oxisol, that has high P content adsorbed in the soil by the internal sphere complex (unavailable for plants).

Carmo et al. [23] and Siqueira Neto et al. [25] provided a comprehensive characterization of GHG emissions associated with the use of vinasse and filter cake as organic fertilizer application practices for planting and regrowth of sugarcane were commonly used in Brazil. Carmo et al. [23] reported significant differences in daily fluxes from soils with organic fertilizers and those with no fertilizer (organic or mineral) (**Table 1**). Daily fluxes from soils that included the application of filter cake and vinasse in combination with mineral fertilizer were significantly

increased in comparison with those observed in the treatment that included only mineral fertilizer. Cumulatively, the highest emissions were observed for ratoon sugarcane treated with vinasse, especially as the amount of crop residue on the soil surface increased. Normally, the flow of $CH_4$ is variable, indicating the ability of the soil to serve either as source or as sink of this GHG [53]. In general, filter cakes can be associated with a lower emission factor compared with other organic or synthetic fertilizers [25]. In turn, the vinasse application can increase $N_2O$ emissions from sugarcane soils, especially during the first couple of days after application [26, 53]. The applied vinasse generates a high emission factor analogous to the emission factor observed for urea application.

Another organic fertilizer is sewage sludge. Although sewage sludge is also very lacking in K, it has high levels of P [60]. This organic fertilizer can improve soil's physical and chemical characteristics and can increase sugarcane productivity, acid phosphatase activity, and biomass [61]. These authors also highlighted the beneficial effect of B, Zn, and Cu from sewage sludge in association with available P that provided increase in the stalks production. However, its use requires some care, as there is the possibility of pathogen and heavy metal contamination. The application of sewage sludge may increase the concentrations of As, Cd, Cu, Ni, Pb, and Zn in the soil, and the quality standard established by the legislation for agricultural soils must be respected [62]. However, the incorporation into soils of sewage sludge rich in C has been shown to increase the amount of dissolved organic matter in soils. Dissolved organic matter can facilitate metal transport in soil through formation of soluble metal-organic complexes [63, 64]; in contrast, they are also able to mobilize some heavy metals sorbed from soil or sewage sludge, being the soil organic matter one of the most important solid phases that adsorb heavy metals, such as Cu and Cd in acid sandy soils. Thus, soils amended with sewage sludge display different physicochemical properties, especially in terms of dissolved organic matter in soil, which will affect behavior of metals in soils. The application of sewage sludge can also provide an increase in $CO_2$ emissions in soils [65]. However, the impact of sewage sludge in the environment on the soil microbial community has not yet been reported for sugarcane agriculture.

The incorporation of ecological practices into sugarcane production and management has the potential to arrest and ameliorate the negative effects of monocropping on soil degradation and yield decline. Historically, the production of green manure as a cover or break crop has been shown to improve the physical, chemical, and biological characteristics of the soil for many crops in production agriculture. Schumann et al. [66] published an interesting review of green manuring practices in sugarcane production. However, only recently, the effects of green manure on soil microbial populations, diversity, and activity in sugarcane soils have been reported [67], in which decrease in the total bacterial population in the soil was revealed, while that of fungi and actinomycetes increased. In addition, Ambrosano et al. [68] verified that green manure is an alternative source of N for sugarcane crops and can supplement or even replace mineral N fertilization. Moreover, green manure associated with mineral N fertilizer altered the soil chemical factors, increasing Ca and Mg contents, sum of bases, soil pH and base saturation, and as a consequence decreased the potential acidity.

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N-fixing biofertilizers are useful to economize the nitrogenous fertilizers and to increase the cane yield. N inputs to the soil can naturally occur as a consequence of the metabolism of $N_2$-fixing microbes. Even though $N_2$ reduction by nitrogenases is an exergonic process, the flow of energy generated is very expensive, requiring much ATP; for this region, nitrogenases are inhibited by $NH_3$ [69]. However, in sugarcane, endophytic symbiosis with $N_2$-fixing microbes is known to occur, and they have been reported for more than 25 years [70]. Although biological N fixation is a natural process in sugarcane, it can be optimized by using more specific and efficient bacteria. The multiplicity of beneficial effects of microbial inoculants, particularly plant growth promoters, emphasizes the need for further strengthening their research and use in sugarcane agriculture.

## 3. Crop residue and harvest management

Soil residue management focusing in soil quality (conservation) and its energetic use are emerging study subjects regarding the sugarcane crop worldwide. In areas under sugarcane cultivation, different sugarcane harvest systems are commonly applied, such as manual handling with burnt sugarcane (burnt harvest) and mechanical harvesting (green harvest). In Brazil, the world's largest producer of sugarcane, harvest practices for sugarcane are undergoing a change, with the increased introduction of mechanical harvesting. This change is regulated by state legislation. For instance, the states of São Paulo and Goiás, which produce more than half of the sugarcane in Brazil, have similar deadlines to completely change their harvest systems. In these states, sugarcane burning is scheduled to be completely phased out progressively during the next 15 years, depending mainly on land declivity due to mechanization limitations.

Without burning, in average, 8–30 Mg $ha^{-1}$ dry mass of straw is generated [9, 71, 72], which has 54% dry leaves and 46% tops [73]. The average crop residue produced every year is approximately 10 Mg $ha^{-1}$ of material with a C:N ratio of approximately 100 [74], that reflects the presence of lignocellulosic composition in the straw, which accounts for 19–34% lignin, 29–44% cellulose, and 27–31% hemicelluloses [75–79]. This characteristic implies in high recalcitrance of residues, that has slow decomposition rate on soil. Around 30–60% of soil moisture content is kept after harvest [80, 81]. There is discussion regarding the feasibility of sugarcane biomass utilization in the industry versus keeping it in the field to improve soil quality and guarantee the long-term sustainability.

Both practices in sugarcane harvest, i.e., burnt and green harvests, have the potential to influence soil physicochemical, microbiological factors, as well as, soil organic fractions. Sugarcane burning as a preharvesting method is a millenary technique to eliminate all leaves and tops around the sugarcane plant, which helps with manual harvest [82] and transport [83]. However, it is known that burnt harvest has the potential to negatively alter the physical, chemical, and biological soil characteristics [21, 84], to increase GHG emissions [85–87], and to decrease soil organic matter [88]. Moreover, particulate matter and smoke from leaf burning released into the atmosphere represent health hazards [89].

In contrast, the maintenance of sugarcane plant residue as a surface blanket positively affects the physical, chemical, and biological soil characteristics. However, these positive effects cannot be observed if soil tillage operations are considered [28]. Conservation agricultural systems, such as minimal soil disturbance (reduced tillage or no tillage), have been sought as an option to conventional tillage practices in order to reduce production costs and improve the soil fertility status [90]. According to Rachid et al. [91], there are no effects from different levels of sugarcane plant residue on the soil bacterial community. However, the authors reported that the soil fungal community can be impacted, and after 12 months, the community can present different structures among the different levels of sugarcane plant residue blankets. Although the physical and chemical characteristics are important for soil quality and sustainability, microorganisms are the main drivers of the nutrient turnover processes in the soil [16] and of the regulation of many atmospheric constituents, such as GHG. In addition, soil microbes have shown many responses to abiotic soil factors, which are clearly affected by microenvironmental changes [14, 92–94].

The current main information related to the impact of sugarcane harvest management on the soil microbial community, soil physicochemical factors, including labile organic C fractions, and GHG emissions at multiple scales are reported below, taking into account the development of more sustainable sugarcane productions systems.

### 3.1. Burnt harvest management

Sugarcane burning has been used for many years on sugarcane crops, and it is still being used currently. Given that soil microbes represent the majority of biodiversity in terrestrial ecosystems and are intimately involved in key ecosystem functions, such as soil fertility, increased attention has recently been paid to microbial communities present in soils under burnt and unburnt sugarcane. According to Souza et al. [13], the level of microbial-C biomass in the soil is lower in burnt sugarcane systems than in sugarcane harvesting without burning. The authors suggested that microbial-C biomass is a reliable indicator of soil quality for monitoring soils under different sugarcane harvesting systems. In turn, Rachid et al. [15] used a molecular approach to evaluate the effect of sugarcane burning and green harvest methods on the soil microbes in the Brazilian Cerrado, and they showed significant differences on the soil bacterial community and its structure between burnt and green harvest systems, with the *Firmicutes* phylum and *Acidobacteria* classes being the groups most affected by sugarcane burning. In general, significant structural changes of the community were observed, with the burnt harvest management having a greater impact than green harvest management on the native Cerrado soil communities. The authors concluded that due to the great variability of the Cerrado ecosystem, further research is required to confirm these findings with soil samples from different sites and seasons in order to address the impact due to changes in management over the years. Val-Moraes et al. [21] also used a molecular approach to evaluate the effect of sugarcane burning and green harvest methods on the soil microbes, and they showed that liming in the sugarcane burnt system and that green harvest practices affect the soil bacterial community. The authors revealed higher bacterial diversity in sugarcane soils than in native forest soil, with burnt sugarcane soil accounting for a higher richness of unique operational taxonomic units (OTUs) than native forest soil. The authors also observed

similar bacterial communities in green sugarcane and native forest soils, while the bacterial community from burnt sugarcane soil was most distinct from the others. *Acidobacteria* and *Alphaproteobacteria* were the most abundant bacterial phylum and class, respectively, across the different soils, with *Acidobacteria* Gp1 accounting for a higher abundance in green sugarcane and native forest soils than in burnt sugarcane soils. In turn, *Acidobacteria* Gp4 abundance was higher in burnt sugarcane soils than in other soils.

In burnt harvest systems, C, N, and S from sugarcane plants volatilize, although they could return to the soil [12]. However, there is an overall tendency of the burnt straw to decrease soil fertility in the long term. The fertilization associated with burnt straw induced by 59 years in Africa [12] and by 35 years in Brazil [95] resulted in decrease of P, K, cation exchangeable capacity, and decrease in Ca and Mg content. In addition, the soil becomes physically exposed due to decreasing of soil organic matter [96] that has great function to binding polysaccharides, fungal hyphae, and humic substances with soil mineral particles forming the soil aggregates [97] and increasing the availability of nutrients [96], which accelerates the loss of chemical fertility [98]. In addition, the harvest burnt also decreases the stability of aggregate on soil surface [10, 12].

Concerning GHG emissions, Figueiredo and La Scala Jr. [86] reported that burnt harvesting increased GHG emissions by 1484.0 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ compared with the green harvest system. However, the authors emphasized that fertilizer application to the soil can also influence GHG emissions. Azevedo et al. [99] reported that burnt sugarcane harvesting intensifies $CO_2$ and carbon monoxide (CO) emissions. Macedo et al. [100] reported emissions of 6.5 kg $CH_4$ $ha^{-1}$ in sugarcane burning. With increasing introduction of mechanical harvesting, a reduction of 39.3% (from 1.053 to 0.639 t $CO_2$ eq. $ha^{-1}$) of GHG emissions was estimated in the state of São Paulo between 1990 and 2009 [101]. According to Capaz et al. [101], there is an increase on ozone and CO content during the sugarcane harvest season due to the burning technique. In synthesis, comparing both harvest management systems, the burnt harvest system presents higher GHG emissions, which range from 558.5 kg $C_{eq}$ $ha^{-1}$ $y^{-1}$ to 2209.2 kg $C_{eq}$ $ha^{-1}$ $y^{-1}$ more than that produced by the green harvest system [102].

### 3.2. Green harvest management

Green harvest has become a recommended approach for sugarcane harvesting. Studies have shown that the soil microbial community is more abundant, active, and diverse in green sugarcane soil than in burnt sugarcane soil [103–105], which influences positively on the soil physicochemical factors. According to Graham et al. [103], the microbial metabolic quotient decreases with increasing soil depth, with significant increases in microbial-C biomass up to 30 cm of soil depth. In addition, microbial-C biomass was significantly higher in rows than in between rows as well as the bulk density was decreased since the green harvest to foster the increase of soil C status [104].

The light fraction from organic matter is another soil quality management parameter that has a chemical composition comparable to that of plant materials [106] and thus, it may be affected by fluctuations in different management practices. Although it represents a small proportion of total soil mass, it contains a significant part of the total soil C and N, so that

its evaluation can provide an early indication of changes in land use and soil management [107]. Brandani et al. [108] verified that burnt harvest combined with organic management was a strategy for long-term storage of total C and N in the light organic fraction, which were related to the quality (diversity) and quantity (frequency) of organic residue addition [107].

Based on a molecular fingerprinting approach, Wallis et al. [109] showed distinct bacterial communities in sugarcane soil under a crop residue blanket in a burnt harvest system. In turn, Rachid et al. [84] reported effects of sugarcane green and burnt harvest management on soil bacterial communities and microbial functional genes. The authors revealed that changes in the soil bacterial community were related to harvest management systems, while soil fungal communities were more sensitive to changes in the crop residue retention levels, probably due to the use of the crop residue as a substrate [91]. Regarding the microbial functional genes, changes in the community structure of ammonia-oxidizing bacteria (*amo*A gene) were correlated with the C:N ratio in the soil, while no significant correlations were revealed between the denitrifying bacteria community structure (*nir*K gene) and the analyzed soil chemical factors.

As mentioned above, the main characteristic of the transition from burning sugarcane to green harvest is the retention of sugarcane plant residue on the soil surface [11, 12]. The sugarcane plant residue retention is an effective practice to: (i) reduce infiltration and soil loss rates [110]; (ii) protect the soil surface from high temperature ranges [110–112]; (iii) maintain the soil moisture levels [110, 113]; (iv) increase earthworm populations and soil microbial biomass [110], which are responsible for organic matter decomposition [95, 113], increasing carbon stocks in the 0–10-cm topsoil layer [83]; (v) increase soil stability and help spread micro and macroaggregates in the soil, which are important for maintaining the soil microbial diversity through the conservation of their microhabitats [12, 98]; and (vi) reduce the necessity of weed control [110]. Hence, green harvest can improve the soil structure and increase sugarcane yield [104, 110] and decrease soil erosion losses [10].

Studies have shown that green harvest may be related to decreases in soil porosity and increases in soil compaction as a consequence of the traffic from harvesters [114], being therefore limited with regard improvement of soil physical factors such as soil bulk density and penetration resistance [98], which could influence negatively on the initial development of root systems, as well as the nutrient availability for plants. However, increases in soil organic matter content and improvements in soil aggregation can gradually reduce the soil compaction [110]. Due to the trend for equilibrium in soil organic matter accumulation, deep drainage and increased soil moisture can promote N losses and denitrification even at low rates [113]. However, the increase in soil carbon by crop residue retention during the ratoon cycles can be lost during tillage operations during the sugarcane replanting period [87], inducing similar soil carbon concentrations for burnt and green sugarcane systems [113].

Nutrient recycling is one of the main reasons for maintaining straw in the field [105]. However, in the first year of sugarcane production, only approximately 20% of the crop residue is available for mineralization and then for denitrification and nitrification, resulting in $N_2O$ emissions from sugarcane plant residues of 71.61 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ [115]. Nitrous

oxide emissions of 420 kg $CO_2$ eq. $ha^{-1}$ were estimated when the total N in crop residue and default values were considered [100]. Because of the high C:N ratio of sugarcane residue, which can range from 70:1 to 120:1 [22], the soil N immobilization should occur in the first phase of straw decomposition. Nevertheless, because gradual availability of others macro and micronutrients from straw decomposition a decrease in $N_2O$ emissions is expected [116]. Fortes et al. [90] observed in a long-term study developed on an Oxisol, that the amounts of straw nutrients released to the soil-plant system (in kg $ha^{-1}$ and in percentage of initial content) were of 12.7 (31%) of N, 0.7 (23%) of P, 43.1 (92%) of K, 18.2 (54%) of Ca, 8 (70%) of Mg, and 4.6 (65%) of S, after the three crop cycles.

Concerning $N_2O$ emission, Pitombo [26] showed that amounts of crop residue from 0 to 11.3 Mg $ha^{-1}$ progressively reduced annual $N_2O$ emissions from sugarcane soils, despite that the highest gas fluxes were verified in the treatments with more residue accumulation (**Table 1**). Nevertheless, the effects of crop residue on $N_2O$ emissions are still unclear in sugarcane soils. Siqueira Neto et al. [25] did not find differences in $N_2O$ emissions from treatments without or with 15 Mg $ha^{-1}$ of sugarcane residue on the soil surface. Nitrous oxide fluxes seem to be higher when crop residue is combined with inorganic N [20, 26, 33]. However, only small areas in sugarcane fields receive inorganic fertilizer, while the majority of the field is important to the $N_2O$ balance [26].

In the first years after conversion from burnt to green harvest, the N fertilizer dose applied to green sugarcane is approximately 30% higher than in burnt sugarcane, increasing GHG emissions by 27% in comparison with burnt sugarcane [99, 100]. Over the years, more crop residue is added to the system, increasing the quantity of readily decomposable organic matter and decreasing N fertilizer inputs [12].

GHG emissions due to fossil fuel consumption of green harvest are related to the diesel use in sugarcane agricultural devices and trucks during the mechanical harvest and stalk transportation [117] (**Figure 1**). They account for nearly 300 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ during harvest operation, with a mean diesel consumption of 74 L $ha^{-1}$ $y^{-1}$ for a 5-year crop cycle [85, 86]. Considering diesel consumption during extraction, processing, and distribution, the GHG emissions increase from 466 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ (in burnt sugarcane) to approximately 750 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ in a 6-year crop cycle [116].

Green harvest results in a total $CO_2$ sequestration of 1173.3 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$ [99, 100]. However, Acreche et al. [118] reported 43% more $CO_2$ emissions from tillering in the green harvest system and 247% more $N_2O$ emissions from post-fertilization than in burnt sugarcane, and the authors reported meaningful $CH_4$ emissions rates compared with those of $CO_2$ and $N_2O$.

Although green harvest showed high GHG emissions due N fertilizer application and fossil fuel consumption, in the first years of the conversion, reduction in the emissions is expected. According to Panosso et al. [112], $CO_2$ emissions were 32% greater in burnt sugarcane, even 7 years after converting to a green harvest system. In the first years after conversion from burnt to green harvest, Figueiredo and La Scala Jr. [85, 86] reported emission reductions of 310.7 kg $CO_2$ eq. $ha^{-1}$ $y^{-1}$, excluding soil carbon sequestration resulting from the crop residue retention.

## 4. Considerations

The large-scale planting of sugarcane crops in tropical regions brings risks that include a potential impact on the soil ecosystem sustainability. To be precise, these environmental risks begin from changes in the soil microbial community, soil physicochemical factors, and GHG emissions from the land use conversion to sugarcane fields. After a relatively long time of fertilizer applications and recycling crop residue on an annual and cyclical (plant stage and ratoons) basis, the ability of the sugarcane soil to maintain its potential for self-regulation in the long term, i.e., its sustainability is threatened. Nutrient management, soil amendments, crop residue, and harvest management in sugarcane soils affect soil microbes at multiple ecological levels, i.e., biomass, community structure, abundance and composition, and taxonomic and functional groups. Consequently, biogeochemical processes mediated by soil microbes are also affected, disturbing the GHG emissions from the soil to the atmosphere in these sugarcane agricultural areas (**Figure 1**).

It is understood that sugarcane renewal is a critical stage for disturbance of the soil ecosystem, in which soil microbes and GHG emissions are affected by soil tillage. Hence, new sugarcane varieties able to delay the need for renewing their planting can cooperate to mitigate belowground atmospheric risks of sugarcane agroecosystems for tropical soil sustainability. In addition, it is recommended to develop technologies for renewing sugarcane cultivation which are able to avoid severe impacts to the soil environment. It is also necessary to enhance farmer access to nitrification inhibitors and controlled-release fertilizers, which have a small market share because of high prices. Although more attention must be devoted to understanding the combined effects of nitrification inhibitors and organic fertilizers on soil microbes and GHG emissions, especially in warm tropical soils, the importance of these products has increased due to the agronomic and environmental benefits already revealed.

In addition, new efforts are needed to quantify the effects of land use changes in sugarcane agricultural fields in tropical regions as well as the effects of nutrient management, soil amendments, crop residue, and harvest management in these agricultural areas on soil microbes and GHG emissions, also taking into account the microbial interactions with physical and chemical factors. Nevertheless, our chapter provides clear signals of the predictable nature of the soil microbe, soil physicochemical factors including labile fractions from soil organic matter, and GHG emission responses to agronomic practices in sugarcane agriculture, which can be used to conceptualize future studies on the understanding of human decision-making for tropical soil sustainability.

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Chapter 2

# Sustainability of Ethanol Sector in Brazil: A Multicase Study

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Additional information is available at the end of the chapter

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**Abstract**

Sustainability has been worldwide discussed, and when it comes to sugarcane-ethanol production, there are many standards required from importing countries to its suppliers. Brazil is the second biggest producer of ethanol in the world, and it has a competitive advantage over other countries since it has a high established production and land for agricultural expansion. The aim of this research is to evaluate the situation of ethanol plants in Minas Gerais state concerning their compliance to sustainability criteria and to briefly present the current situation of ethanol plants in Brazil. This evaluation is based on Bonsucro certification. A multicase study, composed of four sugarcane-ethanol plants, was conducted. The results indicated that the studied plants were not prepared to receive Bonsucro certification. They justified it due to constant changes in legislation relating to sustainability, and the fact that these criteria are not required by their customers, however, was concluded that the companies did not take a proactive posture, seeking knowledge about standards related to the topic. Obtaining international certification did not look appealing by the time of the study, but attendance to national legislation is mandatory. In 2017, the number of certified plants has increased, showing that there has been progress.

**Keywords:** sustainability, ethanol plants, sugarcane, Bonsucro certification, Minas Gerais

## 1. Introduction

Discussions about sustainability and its importance to the planet have been intensified since 1990s. In the face of so many climate changes and environmental disasters, concerns that in the future such picture gets worse have led national and international companies and organizations to think about the best way to work with natural resources, to obtain minor environmental impact.

Sustainability is understood as the ability to use natural resources and, in whole or in part, to return them to the planet, guaranteeing a good quality of life for those living in the environment and for those who will live.

The sugar and ethanol trade in the international market has suffered from nontrade barriers, which has restricted the expansion of the sector, mainly in countries under development. These barriers are standards and directives related to sustainability, which have been imposed by importing countries. Examples are European Renewable Energy Directive 2009/28/EC [1], which was published in April 2009, and Bonsucro (Better Sugarcane Initiative) [2] which was last updated in April 2014.

The European Renewable Energy Directive 2009/28/EC deals with the promotion of the use of energy from renewable sources and sets out a number of requirements to be followed by all domestic and international biofuel producers and buyers.

Bonsucro is a worldwide multistakeholder association created to reduce the environmental and social impacts of sugarcane by designing a standard and program to transform the sugarcane-ethanol sector [2]. The adhesion favors the achievement of high standards of sugarcane production, far above what is determined by national environmental legislation.

The number of certified plants by Bonsucro has increased each year. Between 2015 and 2016, membership grew by 4.5%, and the number of Bonsucro production certificates by 24%, with 12 new certified mills, including first certifications in Guatemala, Nicaragua, Dominican Republic and Thailand, more than doubling the countries which have Bonsucro certifications to a total of 9 [3].

In this sense, this document aims to present the situation of sustainability in the sugarcane-ethanol industries of Minas Gerais state, Brazil, using as indicators the criteria required by international legislation, especially those from Bonsucro and the European Renewable Energy Directive 2009/28/EC. The goal is to present how sustainable the ethanol plants are according to those directives, and how much they could and should be. This is extremely important to sugarcane-ethanol plants, since in many moments, they have been held responsible for negative actions against environment and society, and so, the necessity for clarifications and proofs of compliance with standards on sustainability has been increasingly strong. In addition, it aims to demonstrate how the plants evolved in this topic in the last five years.

### 1.1. Sustainability in the sugarcane-ethanol sector

The process of man-nature interaction is exploratory since the first inhabitants, and thinking about this relationship without thinking about the transformation of the environment is impossible. This relationship was intensified and improved until in the eighteenth century the Industrial Revolution that began in England spread, reaching the other countries. This in turn stimulated by the accumulation of capital consolidated capitalism, an economic model that dominates until the present day [4]. Since then, natural resources have been explored intensively and the impacts of this unbalanced degradation began to be perceived in the twentieth century, causing concern to environmentalists and taking on increasing proportions [5].

In this sense, the discussion of environmental sustainability arises from the limitation and misuse of available resources and can be reversed by the use of clean technologies and, above all, by the creation and consolidation of administrative mechanisms of environmental protection [6].

Sustainability is understood as a combination of three pillars, economic, social, and environmental [7], or also as meeting the basic needs of all, giving them the opportunity to meet their needs in the future, aspiring for a better life [8].

Basurko et al. [9] argue that today there is a growing interest in sustainable products, and it is clear that this major shift toward sustainable development is essential for a competitive market. Moldan et al. [10] say that to improve the cost-effectiveness and operational value of environmental policies in the context of sustainable development, efforts should be made to improve information for decision-making by assessing progress through indicators.

A sustainability indicator can be understood as a quantitative or qualitative tool that allows the analysis of changes while measuring and communicating the progress toward the sustainable use and management of economic, social, and environmental resources [11]. According to [12, 13], there is a range of tools developed, which use indicators to measure sustainability. The use of indicators and indexes for sustainability assessment has increased a lot in recent decades, since it is an instrument that simply expresses a complex message, resulting from many factors [14].

A number of papers have been published about sustainability of sugarcane-ethanol production [15–17] and the impact of it on their countries [18–20]. In those papers, it is possible to observe the range of indicators used and the variety of certifications (compulsory and voluntary) that should be followed and attended.

### 1.2. Dimensions of sustainability

#### *1.2.1. Environmental dimension*

The sugar-ethanol sector is characterized by an activity that uses natural resources intensively. It exerts influence in the generation of foreign exchange for the country, through the production of its derivatives, demanding the attention of public and private entities in the environmental sphere, due to the problems of compaction of the soil, burning of the cane, and emission of gases, which intensify global warming and degrade the environment. The advantages are obtained with the mechanical harvesting without burning, due to the straw that is left in the soil [21, 22].

For the variables that make up environmental sustainability and will be taken as the basis of this study, mention should be made of areas for sugarcane expansion, compliance with legislation, treatment of effluents and waste, and mechanization of the harvest. These variables are of great importance when analyzing the behavior of companies, through the presence or absence of environmental practices internally, as well as meeting the Bonsucro certification indicators, the focus of the study.

Within this context, [6] affirm that an attitude based on immediacy still prevails in business actions, where the management of environmental practices only occurs when there is an imposition of the laws and regulations created in the country, so the importance of evaluating these aspects.

*1.2.2. Social dimension*

The socio-environmental theme, mainly on the focus of sustainability, is increasingly inserted in the different types of markets and involves several stakeholders that drive and direct the organizations to management practices and strategies aimed at minimizing the degradation of the environment, as previously mentioned, and social problems [22].

The social dimension seeks the vision of a balanced society, which seeks a new lifestyle appropriate to the present moment and to the future. It seeks economic development coupled with a significant improvement in the quality of life of the world population, that is, greater equity in income distribution, improvements in health, education, job opportunities, etc. [12].

In the sugar-energy sector, the challenges for social sustainability are related to access to land, working conditions, burning and mechanization of the harvest, and training of employees, linked to the qualification of the workforce. All these factors are addressed in Bonsucro and were evaluated in this study.

Rodrigues et al. [23] argue that monoculture farming practices, such as sugarcane, on large tracts of land have been identified as generating inequalities in the countryside, as well as an obstacle to the social reproduction of traditional populations. The absence of an effective legal system of the Brazilian land structure, capable of regulating and limiting the uses of properties, associated with the availability of cheap labor, has contributed to the expansion of monocultures. These authors also discuss the working conditions in the sugarcane-ethanol sector, addressing issues such as workload, wages, health and safety at work, and within this subject, the most discussed point is the manual harvesting of sugarcane.

After the Brazilian Federal Decree n. 2681, 1998, which determines the complete suppression of sugarcane burning until 2018, throughout Brazil, the harvest mechanization was encouraged and, together with this, the concern of governments and unions about the destination of the workforce will be dismissed from the sector with the growth of mechanization [24]. The change from manual harvesting of burned cane to mechanized harvesting of raw cane has major social implications because a harvester performs the work of 80 to 100 cane cutters [21].

Abreu et al. [25] affirm that it is necessary to carry out programs to requalify cutters who have been dismissed from the labor market to reintegrate them into the labor market and adds that, despite this negative social impact, there is also the positive side of improving workers' health and people living in the sugarcane areas.

*1.2.3. Economic dimension*

The economic dimension is often discussed together with the social dimension, as can be seen in European Renewable Energy Directive 2009/28/EC and Bonsucro Certification. Mello et al.

[12] state that economic and social factors are pooled, so that one helps the growth of the other. The economic growth refers to the question of productivity and competitiveness in order to insulate the economy of a particular region in the world market. As the economy grows, people must benefit from better living conditions: health, more education, more housing [26].

According to Ref. [12], the economic variable should be rethought in its macroeconomic sense. This is made possible through more efficient allocation and management of resources and a steady stream of public and private investments of endogenous origin aimed at achieving this new way of growing. Factors such as the decline in protectionist barriers between countries, the difficulty of access to new technologies, external and domestic debts, and the income inequalities of developing countries need to be considered.

As can be seen, these regulations do not evaluate the economic issue with business vision, the economic gains of the company, but protectionist vision and social development.

Thus, countries' demand for policies that aim a sustainable development has become a necessity for many over the past two decades. In addition to the social question, the fact that the process of environmental regeneration does not keep up with the current consumption pattern has led nations to sign agreements that aim a more equitable and less degraded development [27].

Evaluating the economic bias, the sugarcane harvest is a demanding factor for the plants. The production cost of a sugarcane-ethanol plant that manually harvests cane is 20–25% higher than the other that does not. The use of mechanical harvester increases the efficiency of work and reduces costs with labor [21]. However, as discussed earlier, this factor positively and negatively affects the social and environmental dimensions, and within this context, there is a gain of the environment and a loss for the workers.

In general, the greater or smaller financial performance of companies is what will determine the financial contribution destined to actions that aim sustainability. Therefore, the good functioning of the tripod is allied to good economic results that in turn will return to sustainability, thus forming a cycle [27]. So, these practices heavily rely on the financial performance of the industry to move forward.

## 2. Methodology

The study adopted the multiple case study methodology, with unit analysis, to investigate different practices related to sustainability adopted by four sugarcane plants of different sizes and positions in the sugar-ethanol market, previously selected by a primary and general questionnaire.

The research has qualitative character with descriptive nature. According to Ref. [28] when considering the scope of qualitative research, several approaches are possible, among them: case studies (for a deep contextual analysis of few facts or conditions), detailed interviews, and/or interviews with specialists (for information on influential or well-informed people in an organization or community), document review, and so on. In this work, all these approaches were combined to carry out the study of multicase.

### 2.1. Selection of the sample to the multicase (characterization of the plants)

As in Ref. [29], the type of sampling performed can be characterized as nonprobabilistic and subjective. Hair Junior et al. [30] explain that in nonprobabilistic sampling, the selection of elements for the sample is not necessarily done in order to be statistically representative of the population. Instead, the researcher can use subjective methods, such as personal experience, convenience, expert knowledge, etc., to select the elements of the sample.

Within this context, the data collected within a primary questionnaire were organized into groups according to similarities of information, and from these groups were selected four plants to compose the multicase study.

Eisenhardt [31] states that there is no ideal number of cases, but working with 4–10 cases is a good choice and works well. According to the same author, with less than four cases, it is difficult to generate a theory with great complexity, and with more than 10 cases, it is difficult to deal with the volume and complexity of the data obtained.

In addition, meetings were held with experts in the area, the environmental manager, and the executive director of the Association of the Sugarcane Industries of Minas Gerais, SIAMIG, to assist in the determination of the ethanol plants that have participated in the multicase study. The groups formed and the previously selected sample were presented to them to confirm the similarities and heterogeneity between the cases.

### 2.2. Elaboration of the main questionnaire

The questionnaire was prepared based on the criteria required by the Bonsucro certification because this certification has been looked for sugarcane-ethanol plants.

It contained open and closed questions, being the closed questions in the form of affirmations, that the interviewee should evaluate whether or not he agreed and how much he agreed or disagreed with each statement. Response options were organized on an unstructured, five-point scale, ranging from 1 to 5. In the beginning of the questionnaire, what each value represented was explained : 1—Strongly disagree, 2—Partially disagree; 3—I do not disagree, I do not agree; 4—Partially agree; and 5—Totally agree.

The content of this questionnaire was also discussed with the environmental manager of SIAMIG, to verify the adequacy of the indicators used and, thus, to validate the same.

### 2.3. Data collection

In this stage of data collection, the information was obtained through primary data, which refers to the survey research method. A personal interview with the agricultural, industrial, environmental, and administrative managers of the selected plants was carried out. As this multicase study consisted of a small sample, the survey was considered a good procedure for data collection.

During the interview, it was explained to the interviewees that they should interpret the statements and choose the answer that best represented the vision and practice of the plant. At no point was explained what sustainability is and what the requirements of Bonsucro and European Union Directive 28/2009 are.

### 2.4. Comparative analysis of the plants

The data analyzed were presented in the descriptive form, characteristic of the qualitative research, and in graphs [32]. The data of each case were first treated separately, carrying out a detailed description of each case, using the information collected with the questionnaires.

In this part, information obtained on the fulfillment of Bonsucro criteria was presented in a form of radar-type graphics. To obtain these graphs, the answers were separated by criteria (set of indicators), and for each, the percentage of attendance was calculated.

The next step was to perform the comparative case analysis. Each point on the unstructured scale of the main questionnaire corresponded to an index, and each index indicates a level of sustainability, as follows: Fully disagree: 1: Critical; Partially disagree: 2: Alert; Do not disagree or agree: 3: Reasonable; Partially agree: 4: Acceptable; Fully agree: 5: Ideal. With this information, the comparative analysis was carried out.

In this way, it is possible to identify the indicators that are or not met by the plants, and the level of attendance to these indicators. In addition to the mills, the environment manager of SIAMIG, an expert in the area and knowledgeable of all the plants, was also interviewed to obtain an external view of the plant and to compare and verify whether the responses of these mills match or not with reality. He was given the same instructions as the mills. The identity of the cases had to be revealed to the specialist so that he could answer the questions according to what he knows about each one.

## 3. Results and discussion

### 3.1. Multicase study

#### *3.1.1. Case 1*

Case 1 presents an ethanol plant that is part of an enterprise corporation which has its origin in the Northeast region of Brazil and that moved to Minas Gerais state with the intention of expanding its borders. This unit was founded in 2002, with only the ethanol distillery, inaugurating in 2004, and the sugar factory. Located in the *Triângulo Mineiro* mesoregion, it is a 100% national capital company.

Currently, this unit has 1347 permanent employees, with no temporary employees.

This plant has as main products crystal sugar, hydrated and anhydrous ethanol, and the electric energy. Ninety percent of the total sugar produced is destined to the foreign market, and the rest remains in the domestic market. As for ethanol, 100% is distributed in the national market. Crystal sugar contributes to 67% of the company's revenues, and ethanol accounts for 33% of revenues.

In the harvest of 2012/2013, the plant crushed 2,900,000 tons of sugarcane, producing 5.3 million bags of 50 kg of crystal sugar (266,800 tons) and 79,739 $m^3$of ethanol. Sugar production

corresponded to 7.8% of the total produced in Minas Gerais state, which was 3.42 million tons, and ethanol production corresponded to 4.0% of the total, which was 1.99 billion liters [33, 34].

The plant produces an average of 720,000 KW of energy per day, and of that total, 69.5% (500,000 KW / day) is sold to utilities. With this activity, the company was able to implement the Clean Development Mechanism (CDM) project in 2006 and receive a certificate of reduced emissions (CER), known as carbon credits, which are marketed in the Carbon Market [35].

The area of sugarcane harvested in the last harvest was 39,602 ha, and of that total, 12% correspond to the leased area and 88% of outsourced cane, which are distributed among the 60 suppliers that the plant has. This area of activity covers five municipalities in the region, and on average, the distance from the cane plantation to the plant is 30 km.

Sugarcane in the harvest of 2012 showed productivity of 75 t/ha, which is the average of the 2012/2013 harvest for the state of Minas Gerais. According to the Sugarcane Technology Centre (CTC, in Portuguese, Centro de Tecnologia Canavieira), the Minas sugarcane plantations had a significant improvement in agricultural productivity, closing with a growth of 10% over the last harvest. For the total of recoverable sugars, it presented 173 kg ATR/t cane. According to Ref. [36], in the south-central region, it was expected to obtain 135.60 kg ATR/t cane, in 2012/2013 harvest; thus, sugarcane from ethanol plant 1 was above-average quality of region. In the harvest 2016/207, the average of total recoverable sugars for Minas Gerais was 137 kg ATR/t cane.

Attending the stipulated by the agro-environmental protocol for the sugarcane-ethanol sector of Minas Gerais state, since 2014, all plants that have a planting area in land with a slope lower than 12% must have the crop harvest 100% mechanized. Plant 1 had 98% of the harvest mechanized, in 2012, and the other 2% are from areas and places that it is not possible to use machines.

This company affirms that, in addition to the concern with the economic and production scenario, it also prioritizes the preservation of nature and care with the human being, promoting social programs directed to the community, approaching the areas of health, education, and leisure. When asked about the benefits that the plant's activity brings to the place it is located in, employment generation, local economy growth, partnership with the municipality, partnership with schools in the region, encouragement of culture and leisure, and community programs were cited.

Regarding the environmental practices adopted by the company, there is no-tillage, use of industrial coproducts, use of containment boxes in the soil, contour lines, use of straw to cover the soil, water quality monitoring, and collection and disposal of packaging of pesticides.

In the analysis of attendance of Bonsucro indicators, some criteria can be highlighted due to no attendance by the plant, such as accounting for greenhouse gas emissions (GHG), transparency in the sugarcane expansion process, research incentive (relative to the percentage of the payroll reserved for this purpose), impact mitigation plan, and the percentage of hours lost (**Figure 1**). It is important to observe that the left half of the circle (second and third quadrants) corresponds to the environmental dimension, and the right half (first and fourth quadrants) corresponds to the social dimension.

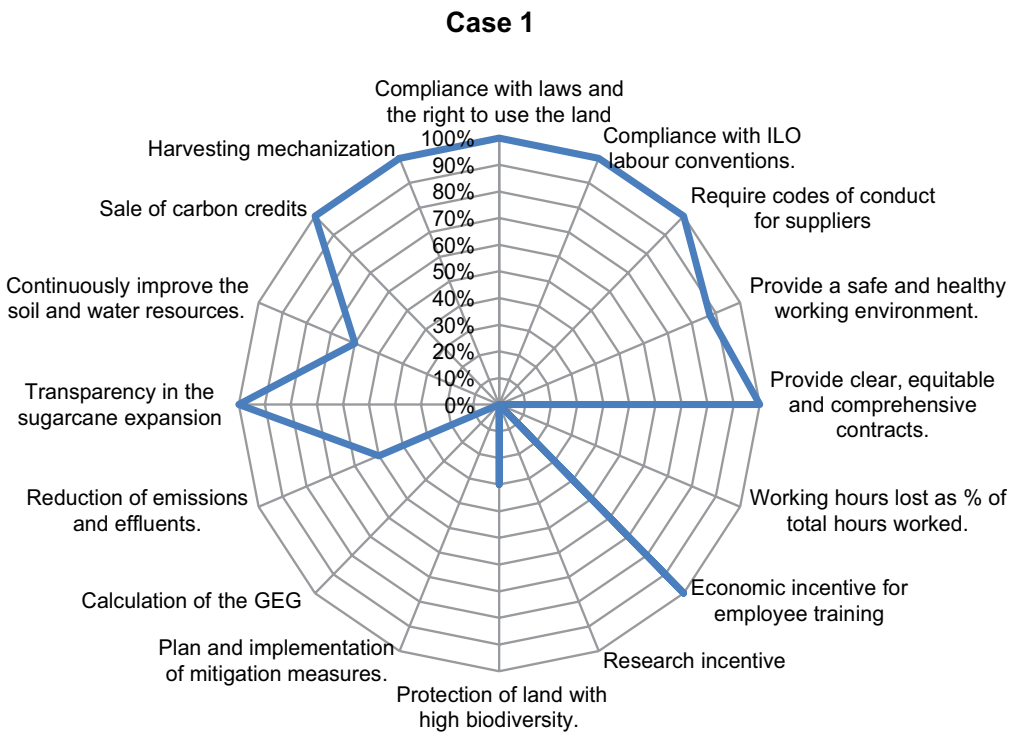


**Figure 1.** Attendance of Case 1 for each criterion of Bonsucro, in percentage.

*3.1.2. Case 2*

Case 2 presents a company that has 100% of national capital and which activities began in 1998. Currently, it has 800 permanent employees, no more temporary because according to the company, with the end of the manual harvest, there is no need of this type of work contract.

In the harvest of 2012/2013, the plant processed 1.1 million tons of sugarcane, producing 2 million bags of 50 kg of crystal sugar (100,000 tons) and 24,000 $m^3$ of ethanol, corresponding to 2.9 and 1.2% of the total production of Minas Gerais state, respectively.

Crystal sugar corresponded to 70% of the production and was fully exported. Only ethanol was domestically marketed. Thus, for the company's revenue, crystal sugar had more relevance than ethanol.

The yield productivity showed good results, 80 t of sugarcane/ha, above the state average. For the total recoverable sugars, 130 kg ATR/t cane was obtained in the harvest of 2012, lower than the average of the center-south region, presented before.

The plant produces an average of 7500 kWh of energy through cogeneration. This energy is not exported (sold) and is fully consumed by the company.

The planting area had 21,733 ha, of which 9051 ha (41.6%) was the plant's own area, and the rest 58.4% are areas of partnerships, that is, leased areas. The mill had no suppliers of sugarcane, and on average, the distance from the sugarcane plantation to the sugar mill was 25 km.

As in the first case, this plant also signed the agro-environmental protocol for the sugarcane-ethanol sector of Minas Gerais state. The plant in question had 98% of its planting areas being mechanically harvested, where the other 2% also corresponded to areas where the machine could not reach or areas of greater slope; thus, the harvest was manual.

When asked about the benefits that the plant activity brings to the region (five municipalities of the northwest of *Triângulo Mineiro*), it cited employment generation with local economy growth, incentive to culture and leisure, incentive to research, and programs and partnerships with universities.

Regarding the environmental practices adopted by the company, the use of industrial coproducts, the use of containment boxes in the soil, contours, use of straw to cover the soil, water quality monitoring, and collection and disposal of packaging of pesticides were cited.

In the analysis of compliance to Bonsucro indicators, Case 2 had a different behavior from the previous plant, and the highlighted criteria for having 0% of attendance were economic incentive for employees training, hours lost at work, research incentive, transparency in the sugarcane expansion process, accounting for GHG emissions, and sale of carbon credit (**Figure 2**). In the same way as the previous case, it is interesting to observe that the left half of the circle (second and third quadrants) corresponds to the environmental dimension, and the right half (first and fourth quadrants) corresponds to the social dimension.

### *3.1.3. Case 3*

Case 3 represents an ethanol plant that was founded in 1986. Like the other cases, it is also a company with 100% of national capital and covers five municipalities of *Triângulo Mineiro* mesoregion.

Currently, it has 1400 permanent employees, no more sugarcane cutters, who made up the temporary employees. With the end of manual harvesting, the plants practically do not hire temporary workers to cut the sugarcane because there is no demand.

The plant produces crystal sugar, ethanol, and energy, and in order of relevance to the company's revenue, sugar is the one that contributes the most, followed by ethanol. As for the distribution of its products in the market, 68% go to the foreign market and 32% to the domestic market.

In the harvest of 2012/2013, the plant crushed 2.35 million tons of sugarcane, producing 3.9 million bags of 50 kg of crystal sugar (195,000 tons) and 62,000 $m^3$ of ethanol, corresponding to 5.7 and 3.1% of the total produced in Minas Gerais state, respectively.

The plant produced an average of 4000 kWh of energy through cogeneration. This energy was not exported, but the company said that the sale of this energy was already in process.

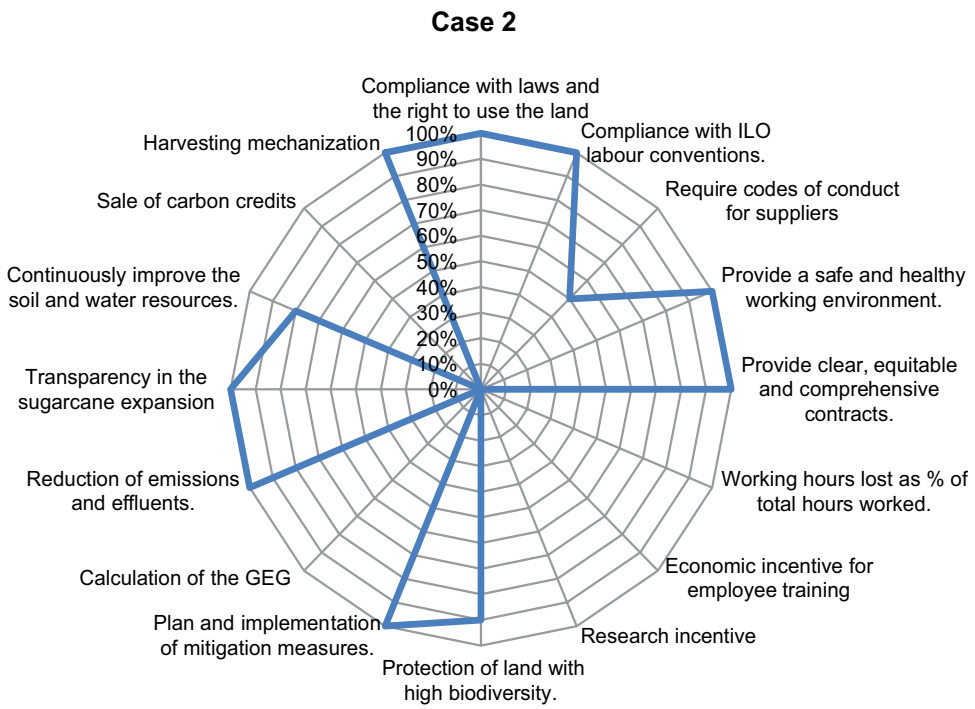


**Figure 2.** Attendance of Case 2 for each criterion of Bonsucro, in percentage.

The area of sugarcane harvested in 2012 was 24,835 ha. This was distributed in leased areas (31.2% of the total), outsourced areas (61.7%), and own area (7.1%). The average radius of the plantation at the plant is 17 km, smaller than the other cases.

The sugarcane yield showed high productivity, 85.8 t/ha, above the national average, but regarding the total fermentable sugars, it presented 123.6 kg ATR/t cane, which was lower than the average of the south-central region in that harvest.

Case 3, as well as previous mills, also signed the agro-environmental protocol for the sugarcane-ethanol sector of Minas Gerais state. Its entire planting area has a slope less than 12%; thus, the sugarcane harvest is 100% mechanized, fully complying the protocol.

This plant, also questioned about the benefits that the activity brings to the region, cited job creation, local economy growth, partnership with the municipality, partnership with schools and universities in the region, incentive to culture and leisure and research encouragement.

Regarding the environmental practices adopted by the company, the use of industrial coproducts, no-tillage, the use of containment boxes in the soil, contours, use of straw to cover the soil, water quality monitoring, and collection and disposal of packaging of pesticides were cited.

Case 3 also presented different information from the other two previous ones, when the analysis of attendance to Bonsucro indicators was done. The criteria that stood out because

they were not attended were economic incentive for employee training, hours lost at work, research incentive, reduction in effluent emissions, transparency in the sugarcane expansion process, GHG emission accounting, and sale of carbon credits (**Figure 3**).

*3.1.4. Case 4*

Case 4 represents one of the oldest ethanol plants in the state, which began its activities in 1920. Located in the mesoregion of *Zona da Mata*, today it is the only survivor in the region of successive crises that the sugar and ethanol industry passed in the last century. Of family origin, the company's capital is also 100% national.

The area of activity of the plant covers 13 municipalities of the mesoregion, which shows the importance of it with job creation and economic growth in the region. It has 1400 permanent employees and 1000 temporary works, differing from the other cases analyzed.

The plant produces crystal sugar, hydrated ethanol, anhydrous ethanol, and molasses, and in order of relevance to the company's revenue, sugar is the one that contributes most, followed by ethanol. As for the distribution of these products in the market, 100% is directed to the domestic market.

In the harvest of 2012/2013, 650,000 tons of sugarcane were crushed, producing one million bags of 50 kg of crystal sugar and 18,000 m3 of ethanol. Comparing with the state production, sugar corresponded to 1.46% of the total and ethanol corresponded to 0.9%. As can be observed among the cases studied, this plant is the smallest.

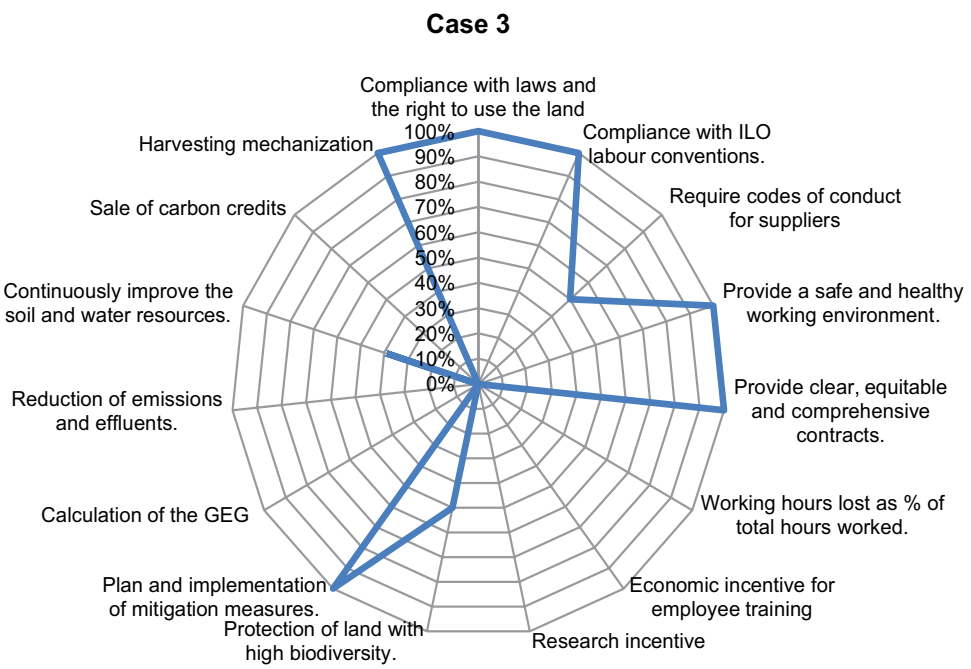


**Figure 3.** Attendance of Case 3 for each criterion of Bonsucro, in percentage.

By burning the bagasse, the plant produces an average of 4500 kWh of energy. This is totally consumed in the company, not being sold.

The sugarcane planted area had 10,800 ha, of which 60% was a leased area and 40% was its own area. Sixteen percent of the total crushed cane comes from suppliers. The average planting radius at the plant is 27 km.

The sugarcane yield had productivity of 70 t/ha, lower than the state average, which was 75 t/ha. In the harvest of 2011/2012, *Zona da Mata* mesoregion was affected by drought in 2012, a fact that justified the fall in sugarcane productivity. Regarding the total fermentable sugars, it obtained 145 kg ATR/t sugarcane, slightly above the Brazilian average, which was 135.6 kg ATR/t sugarcane.

Among the voluntary and mandatory certifications existing for the sector, the plant had only the agro-environmental protocol for the sugarcane-ethanol sector of Minas Gerais state, which is voluntary. This ethanol plant is located in a mountainous region, where 80% of the sugarcane plantation areas of this plant have a slope of more than 12%, so its harvest is 100% manual. Due to the lack of adequate equipment for sugarcane harvesting in areas with declivity above 12%, according to the agro-environment protocol, in projects implemented until 2007/2008, deadlines will be granted for proper adaptation, according to previous authorization of the State Council for Environmental Policy: COPAM.

Like other plants, environmental balance, rational exploitation, and respect for the environment are constant concerns of the company. As environmental practices, it adopts no-tillage, contour lines, water monitoring, and the collection and disposal of pesticide packaging. As social practices, it has partnerships with schools and universities in the region, and with the municipality, encouraging culture, leisure, and research.

In the analysis of compliance with the Bonsucro indicators, Case 4 also had a distinct behavior from the other three plants. As shown in **Figure 4**, the criteria that stood out because these were not met were suppliers' code of conduct requirement, economic incentive for employee training, research incentive, accounting for emissions of GHG, sale of carbon credit, and mechanization of the harvest until 2014.

### *3.1.5. Comparison between cases*

The comparisons were done for each environmental and social dimension presented on Bonsucro and European Renewable Energy Directive. Also, the economic dimension, which is not treated on both certifications, was also assessed and compared between cases.

The first principle of the social dimension in Bonsucro, refers to compliance with the law, and the four ethanol plants affirm to attend completely. In the second principle, which refers to respect human and labor rights, there were some differences concerning their attendance, but all of them had an acceptable level of compliance for the criteria: noninterference of power plants in workers' representation groups; compliance of sugarcane suppliers with labor laws; the use and control of personal protective equipment; and first-aid care.

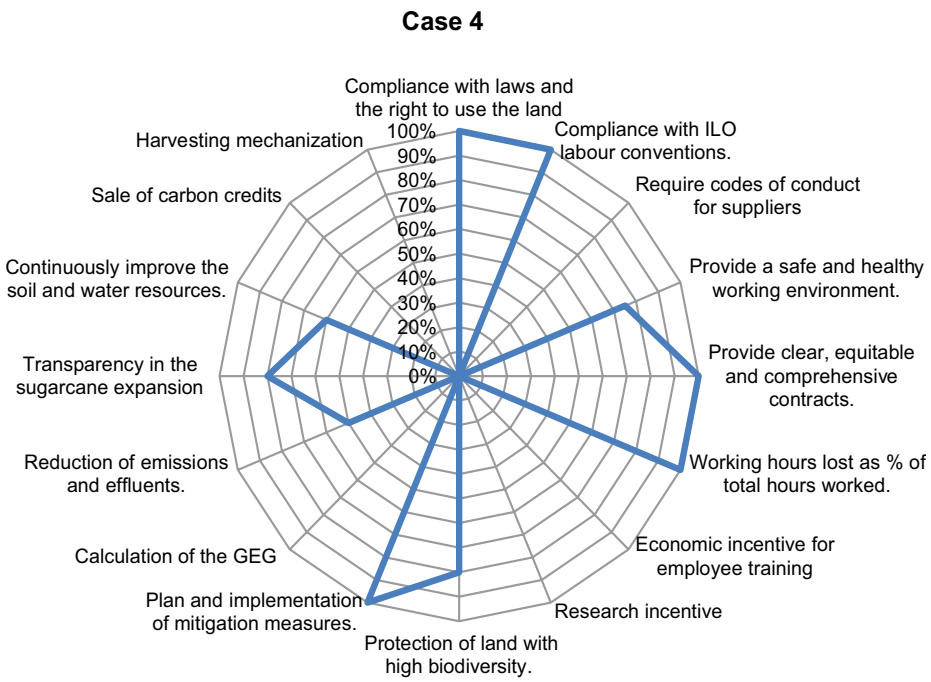


**Figure 4.** Attendance of Case 4 for each criterion of Bonsucro, in percentage.

The differences to be highlighted are the money set aside by mills to invest in employee training and research, which ranges from critical to acceptable; the percentage of hours lost at work; and the requirement of sugar mills to suppliers of codes of conduct, ranging from critical to ideal.

The similarities observed in all four cases, to the environmental dimension, refer to the areas of planting and expansion of sugarcane, which are not areas of high conservation value; the treatment and reuse of waste; the existence and implementation of an environmental management plan; the amount of water captured by industry and agriculture; and finally the practice of no-till. For all those criteria, the attendance ranged from acceptable to ideal. Another observation is that the mills presented better compliance on activities related to biodiversity and ecosystem service management, and the criteria related to Directive 28/2009 of the European Union had more variation, ranging the attendance from critical to ideal.

Other differences found are about the mechanization of sugarcane cultivation, both harvesting and planting, and the sale of carbon credits. It was verified that the plants exploit very little the carbon market and should invest more in the same, since besides the economic reimbursements they also have the environmental benefits, on which the participants of this market are certified by the reduction of greenhouse gas emissions.

About the economic dimension, some financial indicators were used to present the situation of the ethanol plants studied. Case 2 is the one that had the highest growth on revenues (25%), between 2011 and 2013, compared to the others. Case 4 did not present any increase.

The mills were also questioned about the company's profitability increase. According to Ref. [37], from the point of view of owners/shareholders, the most relevant financial measure is profitability, since it reveals the profits obtained by management efforts from the capital invested by the owners. Thus, profitability is the expected return on invested capital. All plants, except Case 4, have increased profitability in the last 3 years, and only Case 1 reported this increase, which was 0.09% a year.

The debt ratio was also used as a financial indicator. This rate measures how much the company is indebted to its creditors, which means the higher this index, the greater the degree of use of third-party capital. Indebtedness indices will more comprehensively visualize the company's financial situation [38]. Cases 1, 2, and 4 had an increase in this rate between 2012 and 2013, being 2.13, 20, and 20%, respectively. Case 1 has the lowest rate, and therefore, it can be concluded that it is the least indebted among respondents. Case 3 did not answer that question.

Among the mills studied, three of them showed an increase in revenues, profitability, and indebtedness rate between 2011 and 2013. It is noteworthy that the mills have used more third-party capital, as well as invested more on their business, and these investments have brought returns to companies. The economic dimension does not have indicators established in Bonsucro or in Directive 28/2009 of the European Union; therefore, the conclusion was based on the interpretation of the data shown here. It can be noticed that there was, in general, economic development in cases 1, 2, and 3 plants studied, but the fourth has been stable in recent years.

### 3.2. The current situation of sugarcane-ethanol plants, in Brazil

From 2012 to 2017, the number of plants certified by Bonsucro in sustainability criteria has increased from 28 to 61 mills [3]. In 2012, there were 26 Brazilian companies certified, and in 2017, this number increased to 43. In Ref. [15], the same behavior was observed in the center-south region, including plants from Minas Gerais state. These scenarios show the commitment of the Brazilian sugarcane industry to incorporate the best social and environmental practices into its agricultural and industrial processes.

The audited area of sugarcane planted advanced from 574,000 ha (or 6% of the total area of sugarcane plantation in Brazil), in 2012, to approximately 910,000 ha of sugarcane in 2017, representing 9.3% of the total harvested area.

The following plants were certified by Bonsucro until 2012: Adecoagro (Angelica; Monte Alegre); Alta Mogiana (Alta Mogiana); Biosev Bioenergy (Sta Elisa); BP Biofuels (Tropical Bioenergy); Bunge (Moema, Frutal, Itapagipe and Guariroba); Copersucar (Açucareira São Manoel, Santa Adélia, Quatá Zilor, Barra Grande of Lençóis Zilor, São José Zilor or Açucareira Zilo Lorenzeti Zilor; Odebrecht Agroindustrial (Conquista, Alcidia and Rio Claro); Raízen (Maracaí, Bom Retiro, Costa Pinto, Jataí, Bonfim, Gasa); Renuka (Equipav); São Martinho Group (Iracema); USJ (São João Araras); after 2012, they were certified: Raízen (Diamond, Destivale, Dois Córregos, Junqueira, Serra, Araraquara, Paraguaçu, Univalem); Copersucar (Santo Antonio Balbo, Uberaba Balbo and São Luiz); Guarani (Industrial Severina, Cruz High

Industrial, Verte: Andrade Sugar and Alcohol); Odebrecht Agroindustrial (Morro Vermelho); Adecoagro (Ivinhema Valley); São Martinho Group (São Martinho and Santa Cruz); Alto Alegre (Junqueira); Companhia Mineira de Açúcar e Álcool (Vale do Tijuco); Serra Grande Group (Serra Grande); Nardini Agroindustrial (Nardini); Not having renewed: Adecoagro (Angelica;); Bunge (Guariroba); Odebrecht Agroindustrial (Alcidia); Raízen (Bom Retiro); Renuka (Equipav); São Martinho Group (Iracema);

In addition to the progress made in relation to the Bonsucro certification, in 2017, the agro-environmental protocol, cited and signed by all four cases, completed 10 years. This protocol was updated, and the main goals are still the end of sugarcane burning; the recovery of forests in springs and the protection of the preservation areas of other watercourses; and adoption of a series of management practices to guarantee sustainability in its production chain. By the year 2022, the plants expect to have completed the process of restoration of all these areas.

As general results for Brazil, we have that 97.5% of the sugarcane area of the State of São Paulo is mechanically harvested and the practice of burning is not applied. This means that since the beginning of the Protocol (2007), more than 9.27 million tonnes of $CO_2$ eq. and more than 56 million tonnes of atmospheric pollutants (carbon monoxide, particulate matter, and hydrocarbons) are not emitted to the atmosphere. The sugarcane-ethanol sector in the State of São Paulo accumulates an asset of 3747 harvesters (between own and outsourced), while in the 2007/2008 harvest, the total was 753. More than 200,000 ha of riparian areas and 8230 springs were protected and recovered; 60% of the signatory plants have forest restoration programs for their sugarcane suppliers; since 2010, the ethanol plants have reduced water consumption by 40% for industrial processing, due to reuse system.

In the last harvest (2016/2017), 131 mills and 25 associations of sugarcane suppliers received the Green Ethanol Certificate as a result of compliance with these actions. These signatories are responsible for approximately 95% of the sugarcane production in São Paulo and 47% of the national ethanol production [39].

## 4. Conclusion

Considering the proposed objectives, to identify and understand the indicators of the Bonsucro certification and the European Union Directive 28/2009, these were achieved when describing the criteria, adapting, and using them as a basis for the research.

Based on the results obtained, regarding the Bonsucro certification and EU Directive 28/2009, we can conclude that none of the plants studied, by 2012, could receive the Bonsucro seal, although some of them present high levels of attendance. Case 3 declared 77% of criteria attendance, despite the external agent declared that it has 38% of attendance.

The fact that none of the plants studied could receive this certification, and the variability between them, called for considerable attention, leading to the belief that all the ethanol plants in the sector, in Minas Gerais state, had followed the same rhythm, being none of them, by 2012, able to get this stamp.

Noncompliance is justified by the plants due to: (1) the constant changes in the legislations related to sustainability, which cause uncertainty and insecurity for mills and (2) the fact that these are not required by their customers. However, it can be concluded that the plants do not adopt a proactive stance, since they do not seek knowledge about norms and certifications related to the subject.

It is important to make a critical analysis of the international regulations that have arisen in recent years in the market. Most of these are based on parameters that are suitable for producers in the countries that gave rise to these standards, making it difficult for producers in other countries to meet these standards, as present throughout the chapter, since each site has characteristics with respect to environment, society, and economy. With this, it is suggested that there is not only a concern with the sustainability, but rather a way for preventing developing countries from growing and expanding their markets.

Despite this, there is still an effort on the part of the plants to comply with the legislation, being aware of their progress and the path that must be taken. As presented, there is a rush and pressure exacerbated by some international entities to meet all criteria and to be sustainable. However, the Brazilian society, its interests, and its criteria are not credited or listened to.

It is believed that sustainability in the sugarcane-ethanol sector should be sought by the plants, not only to meet criteria, but also to improve the sugarcane production system, since this effectively translates into opportunities, biodiversity, and economic gains. If this does not happen, the pace to pursue sustainability will continue to be slow and challenging.

However, if this translates into opportunities, the outputs indicated in this work to meet the sustainability criteria are to: (1) improve the training of employees in all work areas of the plant, explaining to them the importance of the theme, (2) facilitate the diffusion of information about sustainability, (3) organize technical team in the area near the plants to better implement the practices of sustainability, and (4) better control the techniques adopted by the suppliers of sugarcane and inputs, considering that not only the industry, but also the whole sugarcane-ethanol chain must be involved in this process.

Finally, the results obtained in this work indicate that the plants are adapting to the theme, but that several points, such as accounting for the emission of gases and the sale of carbon credits, must still be worked on to improve the situation of the same, regarding the obtaining of certifications. Although these do not seem attractive at the moment, compliance with national legislation is mandatory. It is noted that incorporating the vision of long-term sustainability within organizations is necessary for those who want to be sustainable, certified and expand the market.

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Chapter 3

# Sugarcane Production in China

Muqing Zhang and Muralidharan Govindaraju

Additional information is available at the end of the chapter

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**Abstract**

Sugarcane production in China has a prolonged history since fourth century BC. At present, China is the world's third largest sugar producing country after Brazil and India. During the past decade, more than 90% of the sugar production was contributed by sugarcane. Guangxi is the dominant sugarcane and sugar producer, accounting for 65% of sugar production in China. China's sugarcane production faced serious problems in the past several years, especially the rapid increase in the labor cost because of the manual harvest. Now, China requires changing their sugarcane practice from manual to mechanical in order to catch up with the international trends in worldwide industry. Many other challenges and constraints are becoming severe, including abiotic and biotic stress, cost escalation, over fertilization, poor ratooning, and single cultivar. New technologies will be applied to sugarcane production, including omics-based breeding, best management practices, and so on.

**Keywords:** sugarcane, production, breeding, best management practices, China

## 1. Overall introduction of sugarcane production in China

China is the world's third largest sugar producing country followed by Brazil and India. About 270 sugar mills were working to meet the basic sugar supply in China, of which 233 mills worked for sugarcane, 37 for sugar beet, and 11 for refinery. The sugar industries contributed to GDP (gross domestic product) of about 6–8 billion RMB, about 0.1% of the gross GDP in China [1]. During the past decades, more than 90% of the sugar production was contributed by sugarcane. Sugarcane is a major crop in southern China, especially in Guangxi, Yunnan and Hainan, and western Guangdong. Guangxi is the dominant sugarcane and sugar producer in China, accounting for 65% of sugar production in China.

## 2. History of sugarcane production in China

China is one of the original producers of sugarcane. *Saccharum sinense* and *Saccharum spontaneum* are widely distributed, from the North Qinling Mountains to the South Hainan Island. Since the late fourth century BC, sugarcane has been used to produce syrup in China.

Cane production, cane yield, and quality have been improved very quickly in mainland China in the past 60 years. From 1961 to 2013, sugarcane production increased rapidly from 2.643 million to 126.13 million tons; the total area of sugarcane plantation extended from 0.108 million to 1.827 million ha, cane yield from 24.0 to 67.4 tons per ha and total sugar production from 0.15 million to 10.613 million tons (**Figure 1**). The average sucrose content, over this same period, has increased from under 13% to more than 14.5%, with some cultivars now providing an average over 16% (from October to April).

From that on, the sugar industry in China has suffered four consecutive years of operating losses due to high production costs (labor cost), the elimination of government support prices, and import competition [2, 3]. Total sugar production in MY 2016/17 is forecast at 8.2 million metric tons (MMT) raw value, down 200,000 metric tons (MT) from post's revised MY 2015/16 estimate. Estimated total sugar production is lowered from 2.15 to 8.4 MMT for MY 2015/16. Sugarcane production suffered a major shock when Yunnan, Guangdong, and Hainan provinces announced in September 2015 that they would cancel provincial floor prices for MY 2015/16. As of early March 2016, approximately 90% of sugar manufacturers were operating at a loss according to industry reports, and a number of small mills have been closed. However, China's cane sugar production is expected to increase for the second year in a row in MY 2017/18, with production forecast at 9.2 MMT, up 800,000 MT from the revised MY 2016/17 estimate. This increase is due to a significant expansion in acreage, as higher prices have increased farmer returns and encouraged those to plant more cane (**Figure 2**).

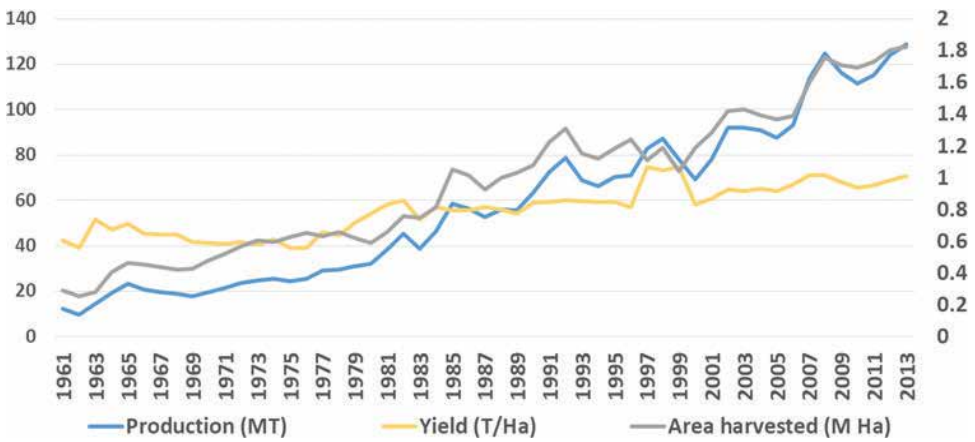


**Figure 1.** Sugarcane production in China (1961–2013).

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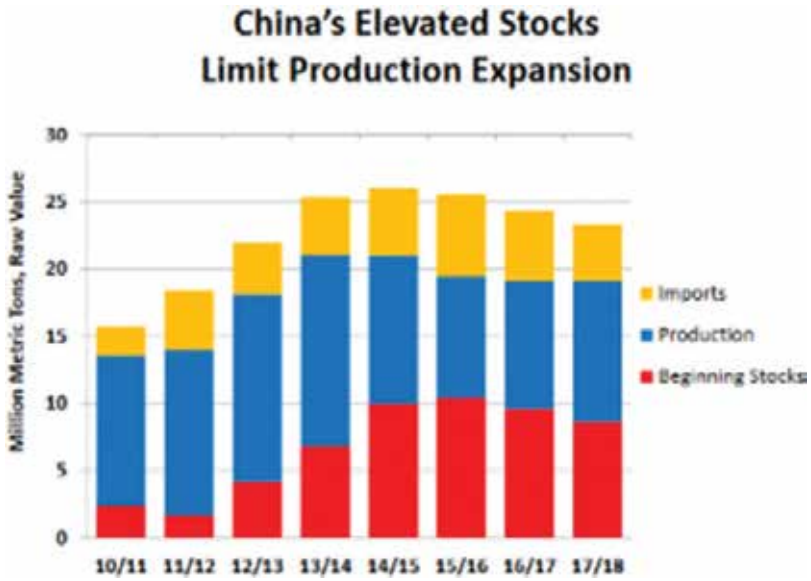


**Figure 2.** Sugarcane productions in China (from 2010 to 2017).

Sugarcane minimum purchase prices (floor prices) are set by local sugar industry associations and sugarcane processors, in consultation with local governments. In Guangxi Zhuang Autonomous Region, the purchase price has increased to 500 RMB ($72) per metric ton of sugarcane, up from 440 RMB in 2015/16 (**Figure 3**). After many years of declining returns,

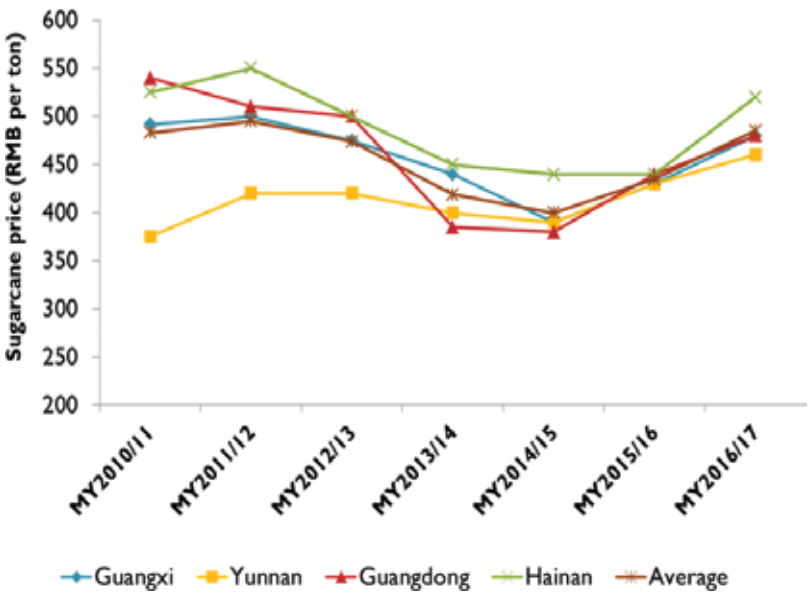


**Figure 3.** Sugarcane price in China (2010–2017).

sugarcane production has become a little profitable, and farmers are consequently planting more (the planting season is typically February and March). Sugar mill contacts have also confirmed that farmers are keeping more of their cane for seed, highlighting this expected increase in area. These increases are anticipated to be greatest in Guangxi, which accounts for over 65% of the nation's total sugarcane production.

## 3. China's major sugarcane production areas

The major sugarcane production area in China is located between latitude 18.5°N and 32°N and longitude 92°E and 122°E, including Guangxi, Yunnan, Guangdong, Hainan, Fujian, Taiwan, Zhejiang, Sichuan, Guizhou, Hunan, and Jiangxi provinces (or autonomous regions). Before the late 1980s, coastal areas in southeastern China such as Guangdong and Fujian were the main sugarcane producing areas. Since then, sugarcane production gradually shifted from southeast to southwest. So, the current major production areas include Guangxi, Yunnan, western Guangdong, and Hainan. The combined sugar production from these provinces accounted for over 90% of the total production in China [4]. Dominant production regions in these provinces were central-south in Guangxi, southwest in Yunnan, western in Guangdong, and northern in Hainan (**Figure 4**).


**Figure 4.** Sugarcane production areas in China.

Despite this increase in area, there are a number of obstacles to continue future acreage expansion. Although a few years ago sugarcane area in China was much more than it is now, this land was planted with fruit trees and eucalyptus trees with a very long production cycle. As a result, even with higher prices, it will not be possible to easily bring this land back into cane production. Mechanization levels are still low in China for sugarcane, and the hilly terrain in much of the production area makes mechanization adoption for harvesting difficult. Production costs continue to be very high. In fact, the price of labor for harvesting sugarcane can make up more than a third of the total cane purchase price [5]. Among major sugar producing countries, China has the highest production costs, with these as much as double those in neighboring Thailand. High production costs and inefficiencies have made Chinese sugar production uncompetitive with other countries, and also it is one of the reasons why the sugar industry approached the Chinese government to request a safeguard investigation into sugar imports for next 3 years (2017–2020).

## 4. Sugarcane breeding in China

The improved varieties are becoming more important for sugarcane production worldwide [6, 7]. New varieties are continuously released in Guangxi, Guangdong, Yunnan, Fujian, and Hainan by sugarcane breeding institutes, agricultural universities, and sugarcane research and development centers in China (**Table 1**). It would be therefore worthwhile for the growers to manipulate the environment in such a way as to bring out the maximum expression of the yield potential possessed by these varieties.

### 4.1. Sugarcane varieties bred in mainland China

Sugarcane breeding program in China started in 1953 when the first sugarcane breeding station was established in Yachen, Hainan (formerly known as Yaxian county, 18°27′N and 109°50′E), where sugarcane can flower in the field. Sugarcane fuzzes from this station were sent to sugarcane research institutes in different provinces (**Table 1**). In general, this station can make 1200 crosses from 1600 flowers every year. Besides this station, Ruili hybrid station (Ruili, Yunnan) can provide about 500 crosses. The number of seedlings is about 0.8 million over the country each year. More than 100 sugarcane varieties have been bred and released for commercial sugarcane production in mainland China from 1953 to 2000 [8]. Of these, GT11 (CP49-50 × Co419), YT57-423 (F108 × F134), YT63-237 (Co419 × CP33-310), and MT70-611 (CP49-50 × F134) have become dominant varieties for a period in different provinces. The combinations and seedling numbers were very limited in China, and the breeding efficiency was very low from 1953 to 2000. For example, less than 50 crosses and 30,000 seedlings were evaluated in the sugarcane breeding program in Guangxi Sugarcane Research Institute before 2001. There were no more new dominant sugarcane varieties bred in mainland China since 1980s when GT11, MT70-611, and YT57-423 were released.

From 2000 to 2016, more than 120 new sugarcane varieties have been released for commercial sugarcane production. Of these, LC05-136 (CP81-1254 × ROC22), GT42 (ROC22 × GT2-66), YT93-159 (YN73-204 × CP72-1210), YZ89-151 (GZ64-137 × NJ57-416), YT00-236 (YN73-204 × CP72-1210), FN41 (ROC20 × YT91-976), GT29 (YC94-46 × ROC22), and GT32 (YT91-976 × ROC1)

| Institute name | Location | Abbreviated Chinese name and prefix of varieties selected at each location | Breeders |
|---|---|---|---|
| Guangxi Sugarcane Research Institute, Guangxi, Academy of Agricultural Science (GAAS) | Nanning, Guangxi | GuiTang—GT | Yang RZ, Wang LW |
| Liucheng Sugarcane Research Institute, Guangxi | Liuchen, Guangxi | Liucheng—LC | Lu WX |
| Guangxi University, Guangxi | Nanning, Guangxi | Zhongzhe—ZZ | Zhang MQ |
| Guangzhou Sugarcane Industry Research Institute (GSIRI) | Guangzhou, Guangdong | YueGan—YG; Yuetan—YT | Qi YW |
| Sugarcane Breeding Station, GSIRI | Yacheng, Hainan | YaCheng—YC | Liu SM |
| Sugarcane Research Institute | Kaiyuan and Ruili | Yunzhe—YZ | Wu CW |
| Yunnan Academy of Agricultural Science (YAAS) | Yunnan | YunRui—YR | Jing YF |
| Hainan Sugarcane Research Center, Chinese Academy of Tropical Crop | Haikou, Hainan | Zhongtang—ZT | Yang BP |
| Sugarcane Synthetic Research Institute, Fujian Agriculture and Forestry University (FAFU) | Fuzhou, Fujian | Funong—FN | Deng ZH |
| Sugarcane Research Institute, Fujian Academy of Agricultural Science (FAAS) | Zhangzhou, Fujian | Mintang—MT | Pan SM |

**Table 1.** The main sugarcane research institutes in different provinces in mainland China.

was the major cultivars released by Chinese breeding program. These achievements were contributed to the increasing seedling scales and the inputs for breeding program. Substantial financial support from the government for the long term provides an excellent chance for sugarcane improvement. In Guangxi, the cross combination number increased up to 500–1000, and total seedling numbers to 100,000–400,000 since 2002. The highest seedling number reached 600,000 in 2012. In Fujian, Yunnan, and Guangdong, the situations are almost same as Guangxi.

### 4.2. Introduced sugarcane varieties in mainland China

A large number of overseas sugarcane varieties have been introduced into mainland China since 1930, such as CP series from USA, Q series from Australia, PR series from Puerto Rico, RB series from Brazil, F and ROC series from Taiwan, China, and POJ series from Philippines. After quarantine, most of them have been used as parental clones in the breeding program in China. However, some introduced varieties were suitable for commercial production in some cane growing areas and adopted directly as cultivars. POJ2725, POJ2878, and POJ2883 were firstly introduced from the Philippines, and Badila from Australia, but only POJ2878 and POJ2725 became major varieties for sugar production in China in 1930s. F134 and Co419 (originally from India) were introduced to the mainland from Taiwan in 1947. F134 became the most popular variety in the sugarcane growing areas in mainland China until early 1980.

Since 1980, the CP and ROC series were used most frequently as commercial cultivars or breeding parents [9]. The most popular and widely used cultivars in mainland China include ROC10, ROC16, ROC22, and ROC25 released by Taiwan Sugar Research Institute in China in 1960–2000, HoCP85-384 by Sugarcane Research Unit in Houma, USA in 2010s. The planting area of these four ROC cultivars has expanded continuously due to their high cane yield, high sucrose content, and adaptation to a range of environmental conditions, which accounted for over 70% of the total planting area of mainland China since 1990. The growing areas of new varieties bred in mainland China accounted for less than 30%. Susceptibility to smut, poor ratooning ability as well as adaptability, and yield stability of these newly bred varieties in mainland China are commonly poorer than those of ROC varieties. However, success in the improvement of sucrose content is partly attributed, at least, to the use of introduced varieties with high sucrose, such as CP and ROC varieties, as parents in China. Many of cultivars bred in mainland China have higher sucrose or higher tons cane per hectare (TCH) than the ROC (Taiwan, China) varieties.

### 4.3. Basic hybridization program in mainland China

China, one of the diversity centers of *Saccharum* complex, is rich in sugarcane germplasm resources. Since 1980s, Chinese sugarcane breeders have collected a large number of wild cane resources from different provinces and overseas and maintained most of these in the National Sugarcane Germplasm Nursery, Kaiyuan City, Yunnan province. Among them, *S. spontaneum* and *Erianthus arundinaceus* are more prominent than other wild species (**Table 2**).

A basic breeding program was established for crossing the local *S. spontaneum* at Yacheng with *Saccharum officinarum* (Badila and other noble cane) in 1953. Several $F_1$ progenies have been released and widely used in the Chinese breeding program, including YC58-43, YC58-47, Ya71-374, and Ya73-512, which in turn have produced a lot of commercial varieties, respectively. In addition, more attentions were paid to the germplasm innovation by exchanging germplasm with other countries, utilizing local wild germplasm collections, such as *S. spontaneum*, *E. arundinaceus*, and *Narenga porphyrocoma*, which were crossed and backcrossed with commercial sugarcane varieties. Some promising clones have been selected from $BC_1$ to $BC_4$ progenies [10].

The genus *Erianthus* is one of the important wild relatives to sugarcane and has attracted considerable interest from sugarcane breeders worldwide for many decades. Within the genus *Erianthus*, most species including *Erianthus arundinaceus*, *Erianthus fulvus*, and *Erianthus rockii* have many superior traits for sugarcane improvement, such as high biomass, vigor, ratooning ability, tolerance to abiotic stresses caused by drought and water logging, and resistance to biotic stresses arising from various pathogens and pests [11]. In order to transfer desirable traits from the genus *Erianthus* into sugarcane, sugarcane has been hybridized with the genus *Erianthus* in China since 1990. In general, *S. officinarum* was usually used as female parent for speeding up the nobilization progress of *Erianthus*, and a series of intergeneric $F_1$ hybrids between *S. officinarum* and *Erianthus* have been obtained successfully. However, the resulting $F_1$ progeny could not be backcrossed directly to sugarcane as male parents due to pollen sterility [12]. Hence, the $F_1$ progeny between *S. officinarum* and *Erianthus* were used as female parents to backcross with sugarcane, and a number of

| Genus | Species name | Number |
|---|---|---|
| *Saccharum officinarum* L. | *S. officinarum* | 32 |
| | *S. barberi* | 3 |
| | *S. sinense* | 25 |
| | *S. robustum* | 6 |
| | *S. spontaneum* | 690 |
| | Local cultivar and fruit cane | 96 |
| | Oversea sugarcane cultivars | 665 |
| | Domestic cultivars | 686 |
| *Erianthus* Michaux. | *E. fulvus* | 63 |
| | *E. rockii* | 51 |
| | *E. arundinaceum* | 290 |
| *Narenga* Bor | *N. porphyrocoma* | 11 |
| *Miscanthus* Anderss. | *M. floridulus* | 2 |
| | *M. sinensis* | 31 |
| *Imperata* Cyr. | *I. cylindrica* | 23 |
| *Pennisetum* Rich. | *P. schumach* | 6 |
| | *Pennisetum* spp. | 2 |
| | Total | 2682 |

**Table 2.** Sugarcane germplasms conserved in the National Sugarcane Germplasm Nursery, (Kaiyuan City, Yunnan; 103.23E, 23.70N).

backcrossing lines have been successfully generated in the past 20 years. *S. spontaneum* was used as a bridge species for the introgression of *E. arundinaceus*, due to *S. spontaneum* with a good source of incorporating fertility in order to overcome the pollen sterility of $F_1$ progeny between *Saccharum* and *Erianthus* [13]. So far, a series of fertility $F_1$ progeny between *S. spontaneum* and *Erianthus* had been obtained. Verification of the introgression of *E. arundinaceus* lineage into sugarcane is an essential way in sugarcane improvement. Over the past two decades, a number of genuine intergeneric hybrids between *Saccharum* and *E. arundinaceus* have been verified and patented using sequence-tagged microsatellite site (STMS), 5S rDNA sequences, 45S rDNA sequences, and inter-Alu sequences [14, 15]. Genomic in situ hybridization (GISH) has been used to identify genuine intergeneric hybrids between *Saccharum* and *E. arundinaceus* and to track the introgression of *E. arundinaceus* lineage into sugarcane. In addition, to detect the chromosomal rearrangement between *Saccharum* and *Erianthus* in the intergeneric high-generation progeny [9, 16], some promising clones (BC2-BC5) have been bred from the cross between *Erianthus* and *Saccharum*, such as YC04-55, YC05-64, YC05-164, YC06-92, YC06-140, YC06-166, YC07-65, YC07-71, YC07-74, YC06-111, YC06-61, YC06-63, YC06-91, and YC05-150.

### 4.4. Selection program in mainland China

The selection programs conducted in China are almost the same in all research institutes or universities in mainland China. Clones are first tested in an experiment station at each institute or university for 5–6 years, followed by testing at multiple sites (regional trial) outside the original institute for 4–5 years. In addition to selection schemes operated by each individual institute, China began a new project to evaluate sugarcane varieties in a nationally coordinated series of trials in 1996. The project was named the National Sugarcane Variety Cooperative Regional Test (NSVCRT) and was coordinated by Fujian Agriculture and Forestry University and supported by Crop Variety Examination and Approval Committee, Ministry of Agriculture in China. Till now, a total of 141 sugarcane varieties were tested in the nationwide regional test and 89 were determined for production test in 14 sites from three sugarcane major ecological zones, *viz.* the Southern China Inland Ecological Cultivation Zone, the Southwestern Plateau Ecological Cultivation Zone, and the Southern China Coastal Ecological Cultivation Zone (**Table 3**) [17]. The released cultivars were evaluated on sucrose content, tillering capacity, yield potential, good field appearance, slight thick and long stalks,

| Variety name | Female parent | Male parent | Identified by | Released year |
|---|---|---|---|---|
| GT91-116 (GT19) | ROC1 | YC85-55 | National | 2005 |
| GT93-103 (GT23) | ROC1 | YC71-374 | National | 2005 |
| GT94-116 (GT24) | GT71-5 | YC84-153 | National | 2005 |
| GT96-44 (GT25) | CP72-1210 | YC71-374 | National | 2005 |
| GT96-211 (GT26) | Pindar | GT11 | National | 2007 |
| GT86-267 (GT16) | YT59-65 | Ya72-399 | National | 1999 |
| GT84-332 (GT15) | HN56-12 | Neijian59-782 | National | 1999 |
| GT89-5 (GT17) | GT11 | YC62-40 | National | 1999 |
| GT94-119 (GT21) | GZ75-65 | YC71-374 | National | 2005 |
| GT90-95 (GT18) | CP65-357 | F172 | Guangxi | 2001 |
| YT89-240 (YT48) | CP72-1210 | GT11 | National | 2005 |
| YT91-976 (YT49) | YN73-204 | CP67-412 | National | 2005 |
| YT91-1102 (YT51) | YN73-204 | YT84-3 | National | 2007 |
| YT93-159 | YN73-204 | CP72-1210 | Guangdong | 2001 |
| YT85-177 | YT57-423 | CP57-614 + CP72-1312 | National | 1999 |
| YT96-835 (YT49) | Co419 | ROC10 | National | 2007 |
| YT96-86 (YT50) | YT85-177 | Zang74-141 | National | 2007 |
| FN91-3623 | CP72-1210 | GT11 | National | 2002 |
| FN91-4621 | CP72-1210 | Zang74-141 | National | 2002 |

| Variety name | Female parent | Male parent | Identified by | Released year |
|---|---|---|---|---|
| FN91-4710 | CP72-1210 | Ke5 | Fujian | 2004 |
| FN94-0403 | CP72-1210 | MT69-263 | National | 2005 |
| FN98-1103 | CP72-1210 | Zang74-141 | National | 2009 |
| FN95-1702 | CP72-1210 | YN73-204 | National | 2005 |
| FN83-36 | CP49-50 | FN57-18 | National | 1999 |
| FN81-745 | YT59-65 | CP36-105 | National | 1999 |
| MT88-103 | Co1001 | YC82-96 | National | 1999 |
| MT92-649 | ROC1 | Co1001 | National | 2005 |
| MT86-2121 | Q641 | CP49-50 | National | 2005 |
| MT92-505 | Co1001 | CP73-1547 | National | 2007 |
| MT99-596 | Co1001 | YC73-226 | National | 2009 |
| YZ85-151 | Gang64-137 | Chuang57-416 | National | 1999 |
| YZ92-19 | Gang64-137 | CP67-412 | National | 2005 |
| YZ89-351 | YC82-96 | GT11 | National | 2005 |
| YZ94-375 | CP72-1210 | YC73-512 | National | 2007 |
| YZ99-596 | Co419 | YC85-881 | National | 2009 |
| FN38 | YT83-257 | YT83-271 | National | |
| FN39 | YT91-976 | CP84-1198 | National | |
| FN41 | ROC20 | YT91-976 | National | |
| GT43 | YT85-177 | GT92-66 | Guangxi | |
| GT44 | ROC1 | GT92-66 | Guangxi | |
| GT42 | ROC22 | GT92-66 | Guangxi | |
| GT46 | YT85-177 | ROC25 | Guangxi | |
| GT47 | YT85-177 | CP81-1254 | Guangxi | |
| GT49 | GZ14 | ROC22 | Guangxi | |

*Variety identified by National means that the variety is approved to plant in main production provinces in order to achieve higher sugar yield. Identified by one province means can only be grown in this province; before extending in other provinces, further testing may need to be done in those provinces.

**Table 3.** Part of new varieties bred in recent 20 years and their parents.

long internodes, nonlodging, nonflowering or shy flowering, erect growing habit, absence of spines on the leaf sheaths, good ratooning ability, less bud sprouting, absence of splits on the stalks, and resistance to local abiotic and biotic stress [18].

## 5. Best management practices of sugarcane

### 5.1. Fertigation and water-saving irrigation practices

The distribution and availability of soil water plays an important role in size and distribution of the root system, and also it induces differences in the capacity of crops to exploit deeper

soil reserves. In general, most sugarcane roots are close to the surface and then decline exponentially with depth, which is approximately 50% of root in the top 20 cm of soil and 85% in the top 60 cm. Thus, the moisture extraction pattern from different soil layers follows the root distribution. The percentage of roots in the top 0–20 cm was 62.0%, 23.4% from 20 to 40 cm, 8.8% from 40 to 60 cm, 4.4% from 60 to 80 cm, and less than 1.4% from 80 to 120 cm. Thus, the moisture extraction pattern from different soil layers follows the root distribution.

Sugarcane requires a large amount of water at about 645-738.6 tons per year (**Figure 5**) and fertilizers at 300-330 kg/ha of N, 90-120 kg/ha of $P_2O_5$ and 300 kg/ha of $K_2O$ (**Table 4**) since it is a long duration crop of 10–14 months in China and since it produces huge amounts of biomass. The water requirement of sugarcane is dependent on its growth phase, 8.3% in the seedling, 21.7% in the tillering, more than 56.9% in the elongation, and 13.0% at the maturity. In southern China, the rainfall is enough for sugarcane growth. However, unbalanced distribution of the rainfalls does not match up with the sugarcane growth stage, so sugarcane always suffers from the drought, especially in the Spring and Autumn. More than 80% of sugarcane requires irrigation in China. In recent years, water-saving irrigation practices are developing fast in China, including spray, microspray, and drip irrigations [19]. The fertigation practices coupled application of this water-saving irrigation with fertilization save a lot of water, fertilizer and labor, and improve fertilizer-use efficiency [20]. The fertilizer concentrations in fertigation practice ranged from 0.1 to 0.2% in the seedling phase and 0.2 to 0.3% in the tillering and elongation phase. No any fertilizer is applied in the maturity phase. Fertigation increased cane productivity by 19.2–56.4% and fertilizer-use efficiency by 90%. It also saved water by 30–60%. In dry upland sugarcane areas, fertigation practices are becoming popular since 2000s.

Compared with the conventional application methods, fertigation practices showed several distinct advantages (**Table 5**), including more even distribution of nutrients in the root area, decrease in the losses of nutrient and water, increase in the uptake of nutrient, less labor, and equipment required.

### 5.2. Healthy seed cane program

Sugarcane is vegetatively propagated for commercial cultivation. Different kinds of planting materials *viz.*, cane setts, settlings, and bud chips are used for raising sugarcane crop. Generally, two-bud setts are used for planting in China, while in some areas, three-bud setts

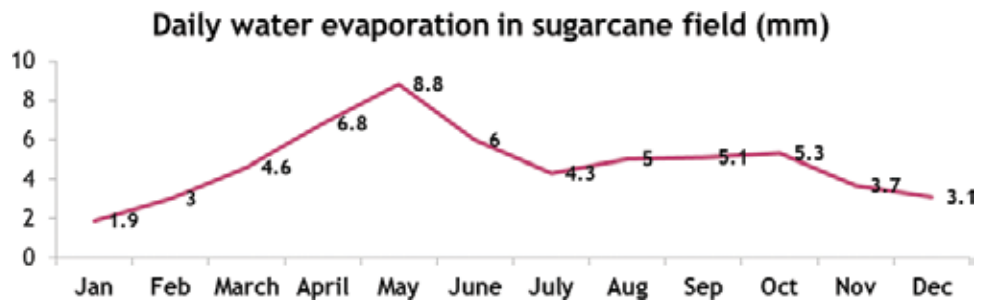


**Figure 5.** Daily water evaporation in sugarcane field (mm).

| Growth phase (d) | Nutrition uptake (g/mu) | | | | | | | Yield (kg/mu) |
|---|---|---|---|---|---|---|---|---|
| | 0–50 | 51–100 | 101–150 | 151–200 | 201–250 | 251–300 | 301–350 | |
| N | 500 | 765 | 2625 | 4750 | 3950 | 2250 | 605 | 13,463 |
| P | 75 | 201 | 665 | 1250 | 835 | 205 | 15 | |
| K | 265 | 535 | 2035 | 7265 | 2503 | 835 | 665 | |

**Table 4.** Nutrition uptake in different growth phases of sugarcane.

are also used. Germination capacity of single-bud sett is very poor due to loss of moisture and fungal or bacterial infection from cut ends on either side. Furthermore, the plants arising from single-bud setts also lack vigor and yield lower as compared to those from two- or three-bud setts. The ideal seed cane involved is as follows: (1) half-year seed cane, (2) fine and viable buds without damage and aerial root, (3) free pathogens and pest, and (4) pure in quality.

Traditionally, farmers in China obtain the seed cane from their harvested cane and have no any treatments before planting, which results in low plant population per unit area consequently reducing the yield. Since 2000, healthy seed cane program has been developed to protect the sugarcane from soil-borne diseases causing pathogens, which usually gain entry into the setts through the cut ends following planting and cause sett rotting and damage to buds, thus affecting germination. The healthy seed cane was produced by micropropagation, thermos- or chemotherapy.

The healthy seed cane was produced at three stages, i.e., breeder's stock, stock seed cane from micropropagation and commercial seed cane. For micropropagation, no pathogen was detected in the plantlets derived from sugarcane stem tip tissue culture, which is required for mosaic, ratooning stunt, and yellow leaf [21, 22]. Use of pathogen-free healthy seed cane improved cane productivity by 15.1–52.1% and sucrose content by 0.12–1.71% due to control of various diseases such as ratoon stunting disease, mosaic viruses, and yellow leaf disease in the seed cane. However, the application of healthy seed cane (about 2% of total sugarcane plantation) was not satisfactory with respect to the production cost, re-infection in the field, and the small-scale farms in China.

Three kinds of major disease (smut, ratooning, and grassy shoot) were transferred through seed cane, which could be eliminated by heat-treatment at 52°C for 30 min and organo-mercurial treatment to protect the setts from soil-borne diseases to ensure better germination. To control

| Efficiencies | Spray | Microspray | Dip irrigation | Control |
|---|---|---|---|---|
| N | 42.33 | 50.39 | 51.32 | 31.46 |
| P | 24.33 | 29.41 | 30.21 | 10.19 |
| K | 41.37 | 51.64 | 52.11 | 27.34 |
| Water | 67.44 | 89.24 | 90.01 | — |

**Table 5.** Differences of nutrition and water use efficiencies among irrigation ways.

the Pokkah Boeng disease, the setts was treated by carbendazim solution at 0.1% (at 1 g/l) for 15 min. To control termites, early shoot borers, and scale insects, the setts was treated by a systematic insecticide *viz.* malathion 50EC (at 2 ml/l) or dimethoate 30EC (at 3 ml/l) for 15 min.

### 5.3. Mechanization for field management

With the urbanization in China, labor is becoming scarce, and labor cost is increasing, thereby favoring mechanization for field management in sugarcane production. In the past few years, the labor cost for harvest increased from 30–50 RMB in 2008 to 130–150 RMB per tonnage. Almost 100% mechanization has been attained in soil preparation and in most field operations such as planting, fertilizer application, mulching with trash and plastic film, and weed and pest controls, but very little mechanization is practiced for sugarcane harvesting (**Table 6**).

For higher sugarcane yields, providing optimum soil environment is an essential prerequisite since the crop remains in the field for about 5–6 years due to the practice of raising several ratoon crops [23]. The ideal land should be prepared by the following steps: (i) subsoiling or chiseling to a depth of 50–75 cm, (ii) discing to shatter clods, (iii) plowing the old crop's residues and organic manures, and (iv) constructing the trench or ridge for draining excess water during rainy season. The land preparation also requires 25 cm of furrow depth and 10 cm of the loosened furrow bottom and drainage channels.

It is necessary to harvest sugarcane at a proper time i.e., peak maturity, by adopting right technique in order to realize maximum weight of the millable canes (thus sugar) produced with least possible field losses under the given growing environment. Otherwise, it will cause great losses in cane yield, sugar recovery, poor juice quality, and problems in milling. In China, more than 95% of sugarcane was manually harvested using various types of hand knives or hand axes. Among the several tools, the cutting blade is usually heavier and facilitates easier and efficient cutting of cane. Manual harvesting requires skilled laborers and large amount of labor cost.

In China, harvesting labor is becoming more scarce and costly in view of diversion of labor to other remunerative work in industry, construction, business, etc. (0.5–1 tons per day for one adult). In addition, more and more younger farmers are not interested in the field operations.

| Operation | Power requirement/ha | | Output (ha/h) |
|---|---|---|---|
| | kW | Diesel (l/ha) | |
| Prediscing | 125 | 18 | 2.5 |
| Ripping | 165 | 48 | 0.5 |
| Plowing | 165 | 24 | 1.7 |
| Postdiscing | 125 | 18 | 2.5 |
| Land leveling | 125 | 7 | 3.5 |
| Ridging | 70 | 16 | 0.5 |

**Table 6.** Power requirement and work output for land preparation of sugarcane.

It is reported that only 5% of younger farmers born in 1990s still worked in the field. Mill stoppages are becoming more common because of nonavailability of canes, which are resulted from the shortage of harvesting labor, especially during Chinese Spring Festival. In addition, most of the new mills are of higher crushing capacity and many old mills are expanding their crushing capacities. Therefore, daily requirement of cane is increasing and hence greater demand for harvesting labor. Mechanization is inevitable and hence, adoption of mechanical harvesting of cane is also inevitable in future.

Compared to the countries, such as Australia, Brazil, USA, South Africa, and Cuba, China has less than 5% of sugarcane harvested by machine. The limitation of mechanical harvesters is use of the large mechanical harvesters in small, irregular and fragmented holdings, diversified cropping patterns, and limited resource capacity of small and marginal farmers in China. In the highly mechanical harvested countries, sugarcane is grown on large plantation scale in large farms owned by either mills or big farmers. The field capacity of mechanical cane harvesters varies with the size (2.5–4 ha per day of 8 h).

### 5.4. Comprehensive control of diseases, pests, and weeds

Sugarcane is a major commercial crop grown in tropical and subtropical regions of China, which is cultivated in about 1.3 million ha. During the last 100 years, the country has witnessed epidemics of various diseases like smut, pokkah boeng, red rot, wilt, and yellow leaf. The damage caused to sugarcane during each epidemic depends upon the nature of disease and spread of the susceptible varieties [24, 25]. The incidence of diseases is increasing at an alarming rate, and the yield is declining every year. About 10–15% of the national sugar produced is lost due to diseases in China [26]. Many sugarcane varieties were replaced due to their breakdown to a new disease or to a new pathogenic strain, such as mosaic, foliage disease, and yellow leaf.

Smut, pokkah boeng, and red rot remains to be the major fungal diseases of sugarcane in China, and *Phoma sorghina* var. *saccharum*, *Alternaria* sp. are the new fungal pathogens causing twisted leaves and brown leaf streak diseases in China, respectively [27–31]. Among the viral diseases, mosaic and yellow leaf syndrome are prevalent in almost all parts of the country. Bacterial diseases like ratoon stunting disease (RSD) are found to cause considerable yield loss in China, while leaf scald disease (LSD) and chlorotic streak disease are also identified to be caused by *Xanthomonas albilineans* and *Xanthomonas sacchari*, respectively [32].

Sugarcane is infested by 287 species of insect and noninsect pests. Out of them, 25 are major pests of sugarcane in China. Borers are major pests attacking sugarcane throughout the growth period, including *Tetramoera schistaceana*, *Chilo infuscatellus*, *C. venosatus*, and *Sesamia inferens*, in which *Tetramoera schistaceana* was the predominant species in China. The sugarcane borer causes the serious economic losses in China by tunneling that enters into the stalk for secondary invaders including bacterial and fungal diseases. More than 25% of sugarcane was infected in China, in some cases reaching as high as 98% of sugarcane. Severe incidences of shoot borer are noticed during water shortage and high temperatures. The other insects include white grubs, wireworms, and yellow sugarcane aphid and mites, including resistant cultivars, biological control agents, insect control and prevention, cultural practices, and

pesticides. A successful integrated pest management (IPM) program helps protect the environment, which also possibly saves money for the growers.

Weeds can reduce sugarcane yields by competing for moisture, nutrients, and light during the growing season. Several weed species also serve as alternate hosts for disease and insect pests. These weed species include Coast cockspur (*Echinochloa walteri*), Goosegrass (*Eleusine indica*), Sorghum-almum (*Sorghum almum*) and *Cyperus rotundus*, etc.

Comprehensive control of diseases, pests, and weeds included resistant sugarcane varieties, pest and pathogen-free healthy seed canes, and green prevention and control practices by integrated managements of physical, chemical, cultural, and biological controls. These green prevention and control practices include as follows: (i) sterilizing seed canes; (ii) removing sources of diseases, pests, and weeds; (iii) using techniques like mechanical trapping in the field; (iv) using pheromone for control of borers, *Trichogramma* and *Cuban flies*; (v) Metarhizium to control termite; (vi) light trapping of borer, longhorn beetle, and scarab; (vii) using herbicide to control pre- and postemergent weed; and (viii) crop rotation for weed control in large scale.

Although chemical and biocontrol methods are effective individually, they are not able to give protection throughout the crop period. If a combination of these agents is available, we can expect a treatment with more efficacy and prolonged protection, such as thiophanate-methyl and carbendazim with bacterial (*Pseudomonas*) for *C. falcatum* [33]. As in other methods of disease control in sugarcane, this approach also works prophylactically. Furthermore, repeated application of the bacterial strains is needed to boost the efficacy.

A network for *Trichogramma* production has been set up in Nanning East Asia Sugar Corporation Ltd., Guangxi. All the cards of *Trichogramma* are provided to all the farmers, which resulted in the decrease of borer incidence by 30% and the increase in sucrose content by 0.5% (absolute value). The best practice program for pest and weed management is becoming a potential and cost-saving approach. Over 70 weed species have developed resistance to the triazine herbicides. These biotypes include several members of the genera *Amaranthus*, *Ambrosia*, *Chenopodium*, *Eleusine*, *Panicum*, and *Solanum*, which are commonly found in China sugarcane fields.

## 6. New challenges and prospects for sugar industry in China

There were about 5 million farms. The average farm size was about 0.27 ha and produced an average of 18 t cane. Most of planting, weeding, cultivation, fertilizing, spraying, and harvesting were still done by hand. Fertilizer was used excessively, especially nitrogen, at three times over the world average, while the usage efficiency was low. It resulted in soil acidification and degradation as well as in pollution. Most of the sugarcane fields were dry slopes with infertile soil. The average available irrigation was less than 20%. Rainfall distribution was uneven and seasonal natural disasters such as drought and frost happened frequently.

### 6.1. Mechanization

In China, sugarcane mechanization is one of the greatest challenges due to the small farm holding and the over requirement of the harvested cane. Most of the sugarcane-growing

areas are lack of over 160 horse powers tractors for soil preparation. Less than 30 cm of plow results in shallow root system and weak soil water-holding capacity, which leads to suffer from drought and lodging.

### 6.2. Over-fertilization

Over fertilization not only increases the production cost, but also leads to low fertilizer utilization efficiency. It is reported that applications of N, P, and K are over 70, 50, and 30%, respectively, of sugarcane requirements [34].

### 6.3. Simplification of commercial cultivar

Cultivars (ROC10, ROC16, and ROC20) from Taiwan Sugar Co-operation were grown in more than 70% of the total sugarcane plantation area, while varieties bred in mainland China were less than 30% in the past 30 years. ROC 22 has been planting more than 50% of sugarcane growing area in China over 20 years. This variety is becoming more susceptible to smut and cold, and poor ratooning ability. Especially, after continuous cultivation of ROC22 in the same fields, the soils have accumulated considerable amounts of smut pathogens. That is why healthy seed cane application for ROC22 does not give expected good results in sugarcane production. Finally, the sugarcane ratooning is limited to 1 or 2 years in China, when compared to Brazil where the production cycle is over 5 years.

### 6.4. Frequent stresses on sugarcane production

Sugarcane production often suffers from the biotic and abiotic stress in China [35, 36]. For example, the frost in 1998–1999 and the drought in 2005–2006 caused serious damage to sugar production. Sugarcane smut, Pokkah Boeng, ratoon stunting, mosaic, and other diseases caused more than a 20% reduction in production. Borers and soil-borne pests (e.g., *Dorysthenes granulosus*, grub) were found in over 60% of sugarcane plantations, which caused the loss of sugar content of over 0.5%.

### 6.5. Prospect and solutions of Chinese industry

CY 2016 is the first year of China's governmental five-year plan (2016–2020) to boost sugar production. The plan's target is to raise annual sugar production to 15 MMT by 2020, when consumption is forecast to reach 18 MMT. The plan also implies the government's intention to gradually reduce imports. Sugar production needs to increase more than 10% annually from 2016 to 2020 in order to meet these challenging policy targets. The government has stated that it will provide subsidies and financial support to farmers to increase yields and reverse declines in sugarcane acreage. The Guangxi government has already started providing subsidies of $5625 per hectares for seeds to sugarcane farmers, farm machinery, mulching film, and fertilizer. The goal is to reach 333,300 ha of "double-high" (high in sugar content and yield) sugarcane. "Double-high" production is classified as a yield of at least 120 MT per hectare and sugar content at 14% or more by constructing the sugarcane production base for optimization of cultivars, mechanization of production, modernization of water conservancy, and operation at large scale [37]. Currently, in Guangxi province, sugarcane production is

under 75 MT per hectare and sugar content is 12%. So, there is a long way and urgent issue to go for sugarcane production in China, including breeding cultivars for mechanical harvest and planting sugarcane at economical and efficient production.

To date, no complete sugarcane genome sequence has been reported, which restricts the development of functional genomics and modern breeding. Omics-based sugarcane breeding will benefit sugarcane production by shortening breeding duration and increasing selection efficiency, including transgene, genomic edit, and marker-assisted selection [38–40]. They have created much enthusiasm to identify genetic components of traits, particularly quantitative traits, in Mendelian factors, and to monitor or direct their changes during breeding through omics-based selection.

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Section 2

# Bionergy and Carbon Sequestration

Chapter 4

# Sugarcane Bagasse Valorization Strategies for Bioethanol and Energy Production

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Additional information is available at the end of the chapter

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**Abstract**

The use of sugarcane bagasse pith as solid substrate for fungi and microbial growth is well known, as well as a source of microorganisms that can be isolated from it. Pith has also been used as a bulking agent for soil bioremediation. More recently, bagasse pith has been used for bioethanol production involving pretreatment and hydrolysis followed by fermentation and dehydration. However, little is reported about biomass valorization for the development of environmentally sound and innovative strategies to process sugarcane bagasse from sugar mills. Incineration of sugarcane bagasse pith is a very common and mature technology for waste disposal and generation of electrical and thermal energy. However, this approach may not be satisfactory in organic waste management due to pollutant emissions, economic and labor costs, loss of energy, and bad odor. In addition, no valuable product is generated from its decomposition process. Instead of incineration, recent research has focused on its utilization as biofuel source. In this chapter, the use of sugarcane bagasse pith as a waste material for incineration versus biomass to produce bioethanol is discussed in terms of energy ratio and emissions, in addition to elucidate the potential of sugarcane bagasse valorization for a more sustainable society.

**Keywords:** lignocellulosic materials, lignin, biorefinery, bioeconomy, heat and thermal power, bio-based chemicals, biofuels

## 1. Introduction

Sugarcane is one of the most widely cultivated crops in the world, with the major producing countries being in the tropics, including Brazil, India, China, Thailand, Pakistan and Mexico.

The extraction of sugar from this crop generates several residues that are often disposed improperly especially where sugar mills use basic process technology. The huge quantities of solid waste are often destroyed or burned inefficiently causing environmental pollution [1]. Sugarcane solid residues include bagasse and filter cake. Bagasse is the solid residue resulting after the juice extraction from the sugarcane stalks and contains the fibrous lignocellulosic material of the stalks. The precipitate in the form of sludge slurry after filtration of the sugarcane juice is the filter cake. Every 1000 tons of processed sugarcane generates about 270 tons of bagasse and 34 tons of cake [2]. Approximately, 1.81 billion tons of sugarcane were produced worldwide in 2015, and this is expected to reach more than 2.21 billion tons by 2024 [3]. Based on these values, the world's potential generation of sugarcane bagasse will reach 0.6 billion tons, which could be valorized into bioenergy, biofuels, and other products.

The expected increase in bagasse availability is driven by the increasing demand for sugar, and sugarcane is the most important source of sugar in the world. However, sugar industries are one of the most polluting ones in view of the generated solid wastes, wastewater, and gaseous emissions of carbon monoxide, volatile organic compounds, and also greenhouse gases during crop cultivation phase [1]. Transforming all by-products obtained from sugar mills (bagasse, filter mud, fly ash and molasses) into value-added products will minimize the pollution to a large extent. Treating sugar industry effluent for reuse in agriculture and other applications is another strategy to reduce the environmental impacts. In summary, sugar industry wastes should be seen as economic resources that can be converted into valuable products in progressing toward resource recovery as a sustainable solution that could generate social welfare and economic development from the sugarcane industry and its residues. In this chapter, the use of sugarcane bagasse as a raw material for energy generation versus bioethanol production is discussed.

## 2. Uses and trends for sugarcane bagasse valorization

Bagasse consists of fibers (48%), water (50%) and soluble solids such as sugars (2%) [4]. Bagasse is an important lignocellulosic material containing cellulose 42%, hemicellulose 28%, lignin 20%, 4.6% of other polysaccharides, 3% of saccharose and 2.4% of ash, on a dry weight basis [5]. Lignocellulosic biomass has been used to produce second-generation ethanol and other by-products such as xylitol by sugarcane agroindustries. Various energy products can be generated from the lignocellulosic composition using biochemical and thermochemical processes. For example, sugarcane bagasse is an economically viable and promising raw material for bioethanol and biomethane production [6, 7]. Bagasse is typically used to produce heat and electricity in sugar mills (cogeneration), but can also be used for paper making, as cattle feed and for manufacturing of disposable food containers. Currently, bagasse is mainly used as a fuel in the sugarcane industry to satisfy its own energy requirements. However, there is a surplus of this bagasse which could be diverted to other uses such as the production of single cell protein, ethanol, enzymes and food additives such as vanillin [8] and xylitol [9, 10]. The sugarcane bagasse surplus is used in more than 40 different applications, including pulp and paper, boards, animal feed, and furfural [11, 12]. **Figure 1** shows some of the various uses of the sugarcane bagasse.

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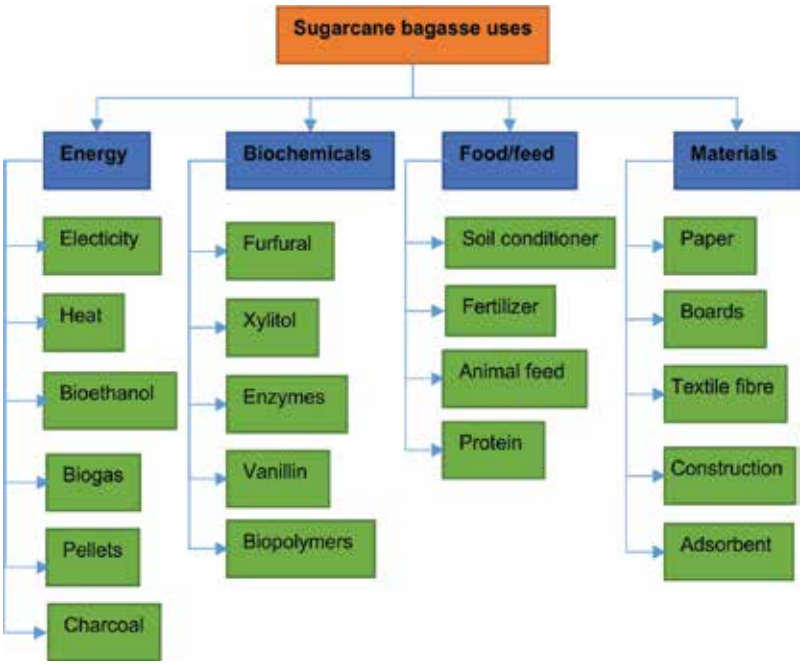


**Figure 1.** Uses of sugarcane bagasse in energy, biochemical, food and feed, and materials applications.

Bagasse is used as a more sustainable source of diverse paper products including toilet, tissue, corrugating medium, news print and writing paper [13–15]. Poopak and Reza described the process of paper making which starts by separating bagasse fibers from the pith by mixing it with water and using a dewatering unit [15]. Fibers are then cooked approximately 10–15 min in a steam boiler, where a black liquor or pulp remains in container. This pulp is washed to remove the color and then sand and undesired fibers are removed by screening and cleaning. Afterwards, pulp thickening reduces the water to about 12%, and it is further processed for whitening the pulp by using chlorine gas and NaOH. This pulp is then ready to supply to a paper mill where the pulp will go through several processes to create paper products. The use of sugarcane bagasse as a renewable raw material can be a sustainable option to reduce deforestation and impacts of the pulp and paper industry.

Charcoal from sugarcane bagasse is another possible source of heating and cogeneration of energy, and can be produced according to the following simplified process [1]. Bagasse is collected and passed through a pyrolysis step where it gets fully carbonized. The resulting powder is mixed with a binding material such as starch and then boiled with water so that it can be extruded to form briquettes or other desirable shapes of charcoal to be sold as a solid fuel.

Recent trends in the use of sugarcane bagasse include new and improved applications in the areas of materials. For example, the bagasse has been used as an excellent soil conditioner to improve sugarcane plant productivity and health [16]. Sugarcane bagasse can also be used for products that improve the durability and mechanical properties of construction materials and as a binder [17]. The bagasse fibers can also be conditioned to be used in the textile industry [18],

and as an effective adsorbent material to remove toxic metals and dyes from wastewater [19, 20]. More recently, sugarcane bagasse has been used as a raw material to produce carbon quantum dots which can be used as biosensors in light-emitting diodes and even in drug delivery [21]. This chapter concerns the two most common applications of energy and bioethanol production from sugarcane bagasse, which are described in the following sections.

## 3. Sugarcane bagasse incineration for energy generation in sugar mills

Burning or incineration in a boiler for steam generation is the most common application of bagasse using a cogeneration system for steam and power generation [22]. This allows supplying heat and power to the sugar and ethanol process and exporting any excess. In countries such as Brazil, where sugarcane industry is well developed, power generation has been largely supported by the government incentives and can be a major revenue component, after sugar and ethanol sales. **Figure 2** shows the two simplified typical cogeneration systems used.

The backpressure steam turbine (BPST) system in **Figure 2a** is more common. In this system, only the amount of bagasse necessary to match the heat required for the process is burned, thus leaving some excess bagasse that can be used for other purposes or needs to be disposed of. The steam is produced from water treated to remove some minerals and is called boiler feed water. The less efficient old systems generate steam at medium pressure of 22 bar and a temperature of 300°C, while the most modern systems can operate at up to 100 bar and 530°C [22]. The steam is then passed through the BPST with a discharge pressure of 2.5 bar and 140°C to meet the low-pressure steam required by the sugar refinery. The condensing

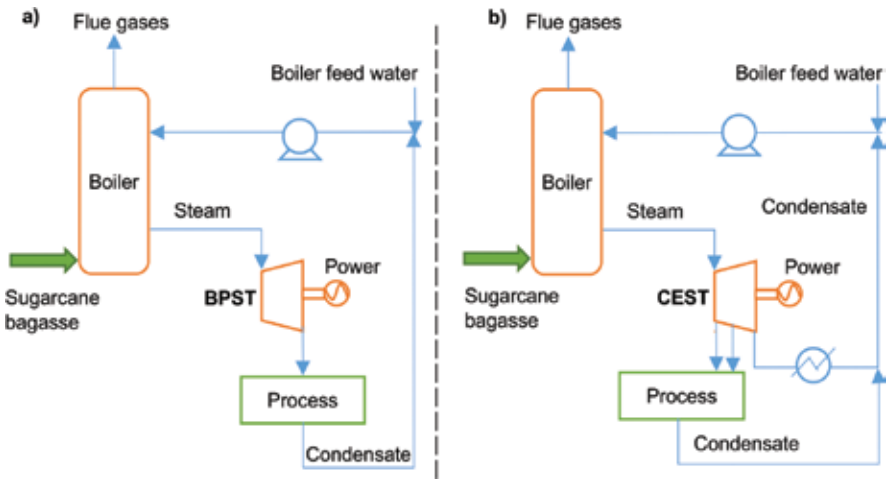


**Figure 2.** Typical cogeneration systems in sugarcane refineries using (a) a backpressure steam turbine (BPST) and (b) a condensing extraction steam turbine (CEST).

extraction steam turbine (CEST) system in **Figure 2b** is more complex and more expensive than BPST, but achieves higher efficiencies and higher electricity surplus, have more flexibility and can operate the whole year. In this system, the high-pressure steam can be expanded at different lower pressure levels and extract the steam required for process or produce further electricity. Typically, the high-pressure steam is at 65–100 bar and can be expanded at 22 and 2.5 bar with a final condensing stage at 0.135 bar.

## 4. Valorization of sugarcane bagasse for bioethanol production

The use of sugarcane bagasse for bioethanol production has been extensively researched in recent years [23, 24]. The processing of sugarcane starts with the cleaning of sugarcane and extraction of sugars: juice treatment, concentration and sterilization [25]. Sugar extraction is carried out using mills to produce a sugarcane juice which follows a series of treatment, clarification and dewatering until the crystallization and centrifugation of sugar crystals. The production of ethanol from the juice, molasses or bagasse includes additional processing units of fermentation, that is, distillation and dehydration.

Ethanol can be prepared by the fermentation of molasses which contain 60% of fermentable sugars as described in [1]. Molasses is first diluted with water in 1:5 (molasses/water) ratio by volume. If molasses lack sufficient amount of nitrogen, it is fortified with ammonium sulfate to provide adequate supply of nitrogen to yeasts. Fortified solution of molasses is then acidified with a small quantity of sulfuric acid. The addition of acid favors the growth of yeasts and hinders the growth of unwanted bacteria. The resulting solution is then transferred to a large tank, and yeast is added to it at 30°C and left to ferment for 2–3 days. During this period, sucrase and zymase present in yeasts convert the sugars in molasses into ethanol according to the following simplified chemical reactions [26]:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

The alcohol concentration in the fermentation broth is only 15–18%. The broth is sent to a distillation system to obtain 92% pure alcohol, also known as rectified spirit or commercial alcohol. A further purification step by molecular sieves or pervaporation is needed to produce anhydrous bioethanol for blending with gasoline.

An additional pretreatment step is needed in the production of bioethanol from bagasse. Pretreatment of the sugarcane bagasse is important because it helps to separate lignin and hemicellulose from cellulose, reduce cellulose crystallinity and increase the porosity of bagasse, thus improving cellulose hydrolysis [27]. Lignocelluloses are made up of three main polymer types: lignin encasing cellulose in cell walls provides rigidity of cell walls, hemicelluloses cover the cellulose and strengthen cell walls by interaction between lignin and cellulose, while encased cellulose microfibrils gives tensile strength to cell walls [28]. Celluloses

and hemicelluloses are polysaccharides of C6 and C5 monomers, respectively, connected by β-(1–4)-glycosidic linkages. The main lignin compounds are polymers of para-hydroxyphenyl (H lignin), guaiacyl (G lignin) and syringyl (S lignin) alcohol. Pretreatment liberates hemicelluloses first because these are hydrolyzed at a faster rate. Liberation of hemicellulose separates lignin and cellulose. β-(1–4)-glycosidic linkages are broken down by pretreatment, liberating glucose from celluloses. The various methods for pretreatment of lignocellulosic materials such as sugarcane bagasse include acid hydrolysis, alkaline hydrolysis, steam or ammonia fiber expansion, organosolv, enzymatic hydrolysis, microwave and ultrasonication, and thereof combinations between these. The most common method is the dilute acid. Ozonolysis has also been used to pretreat sugarcane and agave bagasse [5].

**Table 1** shows values for bioethanol yields reported for various systems. The key to high ethanol yield is to enable the conversion of both hexoses and pentoses into ethanol. This requires the search for new microorganisms and their metabolic engineering. A leading second-generation bioethanol plant using sugarcane bagasse is operating in Brazil by the company Raizen, a joint venture between Shell and Cosan. This highly advanced integrated facility is able to boost bioethanol production by up to 50%, in addition to the first-generation plant and without expanding cultivation land use. The use of bagasse and straws allows production even during off-season for sugarcane harvest. The progressive scaling-up has allowed producing 7 million liters in its first year and planned to reach a groundbreaking 40 million liters by 2018 [29].

Ethanol is used as an alternative energy source in top sugarcane-producing countries such as Brazil, India and China. World production of ethanol in 2013 was about 89 GL, with 74% of the world supply coming from Brazil and the USA [1]. The increasing biofuel production causes an increase in the biomass demand for energy purposes, which poses the challenge of the fuel versus food dilemma. The use of biomass has also raised some questions about the real benefits to decrease environmental impacts of the bioenergy systems that seek to replace fossil fuels due to the greenhouse gas emissions generated during crop cultivation and processing. To avoid unintended consequences and the translocation of issues of using biomass resources, a comprehensive analysis taking into account emissions and externalities related to energy and material consumption in the whole life cycle of sugarcane-based bioenergy systems is essential to ensure their sustainability.

| System | Total electricity production (GJ/t) | Steam production for process (GJ/t) | Total (GJ/t) | Energy ratio (output/input) | Direct process emissions (kg $CO_2$/GJ) |
|---|---|---|---|---|---|
| BPST system | 0.854 | 2.9 | 3.75 | 0.20 | 118.8 |
| CEST system | 1.507 | 2.9 | 4.4 | 0.24 | 101.2 |

**Table 1.** Energy ratio and direct process $CO_2$ emissions for bagasse use in power generation.

## 5. Energy balance and emissions

The current major use of sugarcane bagasse is for power supply in sugar refineries, making this facilities energy self-sufficient. Depending on the process configuration and energy requirements, some of them even export electricity to grid due to the excess bagasse available [23]. As commented in the previous section, an alternative use extensively researched nowadays is in bioethanol production [24]. In this section, the energy balance and emissions of the two alternative uses of bagasse are discussed. The indicator used to compare energy balance is the energy ratio which is defined as the energy output per unit of energy input. Energy input includes the energy originally contained in the bagasse based on its higher heating value. In the case of bagasse for power generation, the only input is the bagasse itself, in the case of the bioethanol production, the input also includes steam and electricity to run the second-generation bioethanol plant.

To perform an energy balance using sugarcane for power generation, it is necessary to know the amount of steam and electricity required for the main sugar factory process. A typical electricity demand is 28 kWh/t cane and the process steam consumption of 500 kg/cane with low efficiency factory, or about 280–340 kg/t cane for modern efficient factories [33]. The balance also depends on the pressure at which the steam is generated and fed to the turbines. Using data from [22], the energy balance of a BST and CEST system on the basis of 1 ton of bagasse is shown in **Table 2**. Current efficiencies are quite low, only 20–24% and, as expected, the CEST system performs better with higher energy ratio and lower $CO_2$ emissions per GJ of energy delivered. These values can be improved further through reduction of steam required in the sugar factory by better energy integration as well as by replacing old equipment with more efficient one. Highly efficient cogeneration systems can achieve up to more than 80% efficiency. Improvements can lead to a significant amount of surplus bagasse becoming available for other purposes such as production of bioethanol. In such a case, approximately 50% of the bagasse is sufficient to supply the energy needs of sugar mills [33].

Given the wide availability of bagasse as an agroindustrial residue, its use for bioethanol production has been widely investigated. The energy balance for this process may be less favorable as the ethanol yields can be relatively low and may require additional energy inputs. Strategies to achieve higher efficiencies in integrated systems combine (1) higher ethanol production can be achieved by the proper pretreatment and hexoses and pentoses fermentation

| System | Ethanol yield (L/t bagasse) | Reference |
|---|---|---|
| Pretreatment + enzymatic hydrolysis | 149.3 | [30] |
| Two-stage dilute acid pretreatment + organosolv | 192 | [31] |
| With pentoses also fermented to ethanol | 335 | [32] |

**Table 2.** Reported bioethanol yields from sugarcane bagasse.

process and (2) the lignin and solid residuals are used for energy production. This can be achieved by adopting a biorefinery concept in which several process technologies are combined to convert biomass into multiple products [28]. A simplified diagram of an integrated biorefinery system is shown in **Figure 3**.

**Table 3** shows the energy ratios reported for several integrated bioethanol processes in biorefineries. It can be observed that an energy ratio of up to 0.5 can be achieved using a simultaneous saccharification and fermentation process, including strategies (1) and (2) aforementioned. Energy integration using pinch analysis is also essential to reduce process utility requirements and increase energy efficiency [35, 37].

It is important to examine the life cycle emissions as the bioethanol process uses additional inputs, including enzymes, nutrients, salts, neutralizers, and so on. An average value of 6.2 kg $CO_2$/kg ethanol has been reported [35]. More comprehensive results of life cycle assessment environmental impacts are shown in **Table 4** for the impact categories of global warming potential (GWP-100 years), abiotic resource depletion (fossil fuels), eutrophication and acidification potentials of the integrated biorefinery system in **Figure 3**. These results show that the amount of GWP can be negative due to the savings by replacing fossil fuels by ethanol and grid electricity by the power generated from lignin and biogas.

Comparing the two options for bagasse utilization, a study shows that the use of bagasse for power generation results in lower global warming, acidification and eutrophication potentials, whereas the bioethanol production provides resource conservation (by replacing fossil fuel) and lower human- and eco-toxicity [33]. In terms of energy balance, with the use of advanced technologies and process integration, both systems are able to achieve high efficiency level up to 50% in the bioethanol case. Up to 65% of the energy from bagasse incineration can be recovered by the biorefinery system in **Figure 3**, while only 32–33% of the energy is recovered by stand-alone bioethanol production [39]. Therefore, the use of multistage steam condensing turbines, efficient boilers, as well as the integrated first-generation + second-generation system with energy recovery from solid residues and biogas from wastewater treatment is highly recommendable to achieve high efficiency levels and environmental benefits from sugarcane bagasse and sugarcane as an energy crop.

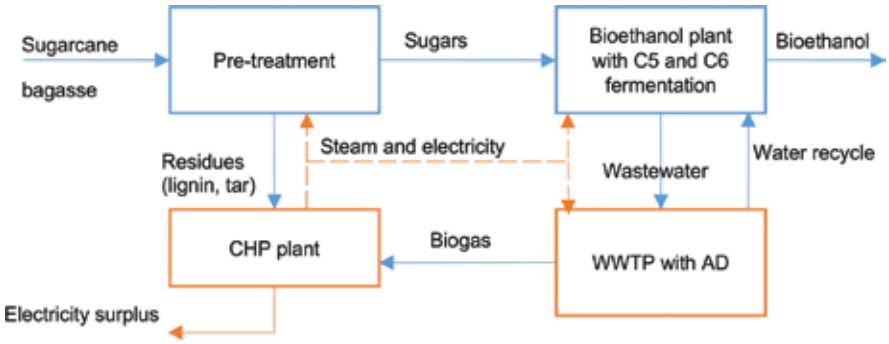


**Figure 3.** Integrated system for bioethanol production from sugarcane bagasse.

| System | Reference | Energy ratio |
|---|---|---|
| Separate hydrolysis and fermentation | [34] | 0.474 |
| Separate hydrolysis and fermentation | [35] | 0.419 |
| Separate hydrolysis and fermentation | [35] | 0.391 |
| Simultaneous saccharification and fermentation | [36] | 0.5 |
| Simultaneous saccharification and fermentation | [35] | 0.438 |

**Table 3.** Energy ratios reported for second-generation bioethanol production.

| Impact category | Abiotic resource depletion (fossil fuels), MJ/t biomass | Global Warming Potential kg $CO_2$-eq/t biomass | Acidification, kg $SO_2$/t biomass | Eutrophication, kg $PO_4^{3-}$/t biomass |
|---|---|---|---|---|
| Value | 1586.16 | −176.29 | 0.46 | 0.03 |

**Table 4.** Life cycle assessment (LCA) results for bioethanol production from sugarcane bagasse [38].

A flexible biorefinery that can adapt production to electricity and bioethanol can be more effective and achieve economic profitability [40]. Integrated first- and second-generation ethanol production process from sugarcane leads to better economic results, especially when advanced hydrolysis technologies and pentoses fermentation are included [32]. Novel ethanol separation and purification processes such as a combination of vacuum, atmospheric and extractive distillation systems for efficient dehydration of ethanol will also help to improve the feasibility of the bioethanol route from bagasse [41]. Other sugarcane bagasse biorefinery concepts have also been studied for production of bioethanol, methane and heat [39], as well as for chemicals, electricity and fuels with succinic acid being competitive in comparison to the petrochemical-based products [42]. Thermochemical processes via gasification and Fischer Tropsch process [23], as well as gasification for cleaner electricity production from syngas has also been reported [43]. A simultaneous economic and environmental impact assessment of biorefinery systems should be performed to enable an informed decision-making as to which process technology to adopt [44].

Although there is no clear winner in terms of energy balance and emissions, the current market has made the use of bagasse for power generation as the focus of some companies to make profits from sales for the grid. Other leading companies, such as Raizen Energy in Brazil, consider second-generation ethanol from the bagasse as a more attractive option [45].

## 6. Final remarks

Sugarcane mills are one of the major industrial facilities in tropical and developing countries, generating income and jobs in the rural agricultural sector. These important industrial systems are evolving from single product process producing sugar to sweeten drinks and food,

to sugar and bioenergy generation in the form of electricity and also biofuels. The valorization of sugarcane bagasse as a resource for energy and bioethanol production has been reviewed in this chapter from the perspective of energy ratio and emissions. Trade-offs between the two bagasse applications have been found with incineration for power generation being favorable toward reducing potential impacts of global warming while bioethanol being more favorable toward resource conservation and lower toxicity. Advanced integrated biorefineries can achieve energy ratios similar to those in incineration for power-only systems, especially if second-generation bioethanol production from cellulose and hemicellulose and electricity from lignin are combined in the sugar mill facilities. Sugarcane mills have the potential to be retrofitted and converted into advanced biorefineries being energy self-sufficient and co-producing other value-added products from sugarcane bagasse in a wide range of applications such as energy, biochemicals, food and feed and materials sectors. Comprehensive energetic, economic and environmental assessment of the various alternative uses and process technologies need to be carried out considering the various efficiencies of the value chain, from cultivation to processing and end use, in order to find the best alternative in a given socioeconomic context.

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Chapter 5

# Competitive Management of Sugarcane Waste and Reduction of $CO_2$ Emissions from Harvest Burning in Supply Regions

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Additional information is available at the end of the chapter

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**Abstract**

Sugarcane is an important crop in more than 100 countries around the world. Their burning is a cultural activity before and after the harvest; however, pollutants and greenhouse gases emitted to the atmosphere can affect the human health and weather, respectively. The aim of this research is to report the $CO_2$ emissions of the main countries dedicated to the cane production and explain their relevant relation with the dry matter available to the burn and how it can affect their alternative uses. The methodology used in this study identifies the relation between biomass burned (dry matter) and $CO_2$ emissions, estimated by the Food and Agriculture Organization of the United Nations with the techniques of the Intergovernmental Panel on Climate Change. The study was carried out for the period of 1990–2014. The results show an important positive trend in the increase in the annual production levels and the biomass burned during the harvest period. The high correlation between harvested area and yield per hectare in countries such as Brazil and the United States allows to have more biomass available for alternative uses. Countries such as Mexico and Colombia have a low correlation between both the parameters due to the increase in the harvested hectares and reduction of their performance per hectare.

**Keywords:** $CO_2$ emissions, biomass burning, sugarcane, harvested area, cane waste

## 1. Introduction

The biomass burning refers to the complete or incomplete combustion of living or dead vegetation by natural or anthropogenic causes [1]. The residual burning carried out worldwide is

emitting a great variety of pollutant species and greenhouse gases such as particulate matter (PM), nitrous oxide ($N_2O$), carbon monoxide (CO), methane ($CH_4$) and hydrocarbons [2], and so on to the atmosphere. The waste obtained from burning agricultural waste occupies the second place in the world (**Table 1**).

One of the crops that contribute to the increase in agricultural residues is sugarcane (*Saccharum officinarum*) and it is cultivated in more than 100 countries around the world [4]. Brazil has the first place in production, followed by India and China, while the United States and Mexico have the sixth and seventh place, respectively (**Figure 1**). This crop has a great economic and alimentary importance, for example, in 2011 it had a world production of about 1.7 billion tons [5], thanks to the variety of products such as sugar, piloncillo, alcohol and food for livestock, and so on. The sugar production and their resulting products depends of the good performance of the crop, which is in function of the saccharose and biomass [6].

The mechanisms of cane harvesting in most of the countries involve their burning before and after the cutting process to remove weeds and to scare animals and insects in harvest area. This crop, well developed, favors the economy and food supply, although it may also contain a great quantity of residues which emit a great quantity of pollutants and greenhouse gases to the atmosphere when burned. In addition, the soil health can be affected due to the loss of important nutrients such as carbon and nitrogen. If these nutrients do not recover, the yield production in the next harvest period can be negative [7, 8].

The sugarcane production per hectare (in t $ha^{-1}$) let to know the countries that more burn this crop in the world due to there is a major quantity of biomass available during the harvest period. In 2009, countries such as Brazil, Australia and the United States had a yield ranges between 65 and 88 t $ha^{-1}$, while in other countries such as Mexico and India had a range between 48 and 65 t $ha^{-1}$ [9]. The biomass burning can also increase if there is an increase in available hectares to plant this crop. In Mexico, this situation occurs in its main cane harvesting regions [10, 11].

| Source of burning | Burning biomass (Tg* of dry matter/year) | Released carbon (Tg* of dry matter/year) | Total proportion of released carbon |
|---|---|---|---|
| Savannas | 3690 | 1660 | 42.1 |
| Agricultural waste | 2020 | 910 | 23.1 |
| Tropical forest | 1260 | 570 | 14.5 |
| Wood for combustibles | 1430 | 640 | 16.2 |
| Temperate and boreal forests | 280 | 130 | 3.3 |
| Carbon | 21 | 30 | 1.0 |
| Total | 8700 | 3940 | 100 |

*1 Tg = 1 × $10^{12}$ g.
Adapted from Ref. [3].

**Table 1.** Annual global estimate of the amount of biomass burned and carbon released to the atmosphere.

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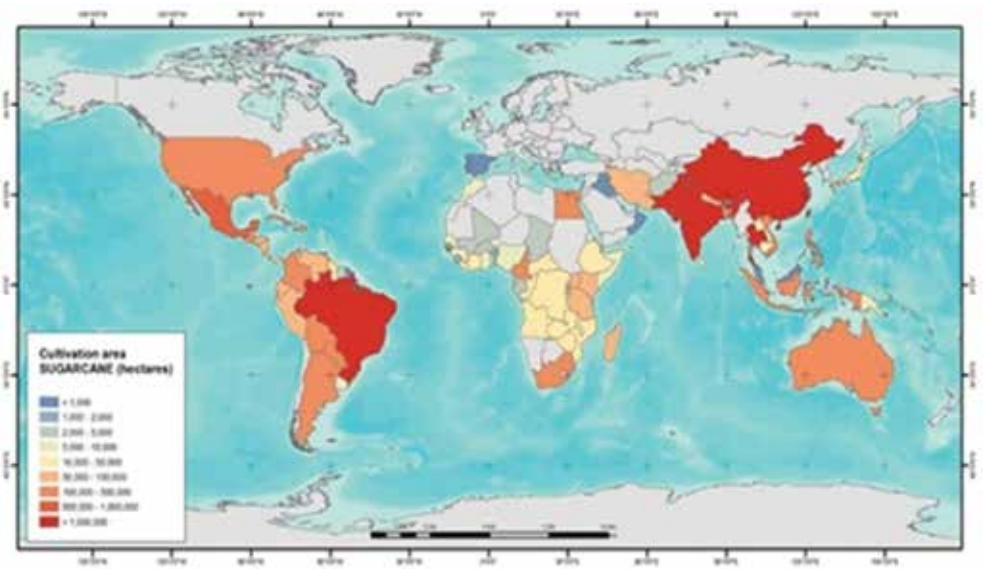


**Figure 1.** Cultivation area dedicated to the sugarcane production worldwide.

The quantity of biomass contained in the crop depends on their development which depends on: geographical, meteorological and edaphological factors [12], related to each other in every stage of their growth [13]; and the cane variety planted (**Table 2**). For example, the efficiency of the photosynthesis process depends on the quantity of solar radiation that affects the leaves of the plants.

The production of the sugarcane is highly correlated with the harvested area as shown in **Figure 2**. There are some countries where the correlation decreases due to diminishing yield levels. For example, in Indonesia, after 2007, while the production declined, the harvested area increased; or in Colombia, where the harvested area increased while the production kept constant. In most cases, there is a positive trend in both parameters in some countries such as Mexico, Brazil, China and India.

| Country | Cane variety | Area cover (%) | Characteristics |
|---|---|---|---|
| Brazil | RB867515 | 26 | High cane yield; excellent performance under mechanized planting and harvesting; resistant to orange rust, brown rust, smut, mosaic; tolerant to leaf, scald ratoon stunting disease (RSD). |
| | RB966928 | 10 | |
| India | Co 0238 | Not available | Subtropical adaptation; high sucrose; high cane yield; nonflowering; nonlodging; moderately resistant to red rot; resistant to smut; tolerant to drought; very good ratooning. |
| | Co 86,032 | Not available | Tropical adaptation; high sucrose; high cane yield; shy flowering; nonlodging; moderately susceptible to red rot; resistant to smut; tolerant to drought; excellent ratooning. |
| China | ROC22 | 54.8 | High sucrose; high and stable ton; poor ratoon; moderately resistant to smut; susceptible to mosaic. |

| Country | Cane variety | Area cover (%) | Characteristics |
|---|---|---|---|
| Thailand | KK3 | 53 | High cane yield; high sugar; good tiller; loose leaf sheet; difficult to flower; poor ratooning if serious drought; moderately resistant to smut and red rot. |
| | LK92-11 | 31 | High cane yield; high sugar content; good tiller; few stalk flower; suitable for irrigation condition; not suitable for sandy soil; resistant smut and red rot. |
| United States | HoCP96-540 | 17.6 | Excellent sugar yield; excellent cane yield; moderate sugar recovery; resistant to mosaic; resistant to smut; resistant to leaf scald, susceptible to brown rust; resistant to orange rust; susceptible to sugar borer; excellent cold tolerance. |
| | CP 89-2143 | 8.6 | High sugar content; moderate cane yield; resistant to brown rust; susceptible to orange rust; resistant to smut; resistant to leaf scald; moderately susceptible to mosaic; moderately resistant to RSD; susceptible to yellow leaf syndrome; no flowering. |
| | L99-226 | 7.7 | Excellent sugar yield; moderate cane yield; excellent sugar recovery; resistant to mosaic; susceptible to smut; susceptible to leaf scald; susceptible to brown rust; resistant to orange rust; resistant to sugarcane borer; poor cold tolerance. |
| Mexico | Mex 69-290 | 25.4 | Resistant to orange rust; brown rust; smut; leaf scald; sugarcane mosaic virus; scarce flowering; mid maturity. |
| | Mex 79-431 | 6.4 | Resistant to orange rust; brown rust; smut; leaf scald; sugarcane mosaic virus; mid maturity; regular flowering. |
| Australia | Q208 | 32.3 | Widely adapted, resistant to brown rust, chlorotic streak, leaf scald, mosaic orange rust, red rot, RSD, smut. Intermediate-susceptible to Fiji leaf gall. |
| Pakistan | HSF-240 | 24.3 | Subtropical adaptation; tolerant to drought and frost; moderately susceptible to red rot; resistant to rust; highly susceptible to smut; resistant to ratoon stunting disease; resistant to red stripe. |
| | SPF-234 | 21.9 | High yielding; moderate to high CCS; highly susceptible to red rot; susceptible to rust; resistant to smut; resistant to ratoon stunting disease; resistant to red stripe. |
| Colombia | CC 85-92 | 52 | High cane yield; medium sugar yield; average self-trashing; adapted to semidry zone; resistant to orange rust, smut, mosaic, sugarcane yellow leaf virus; susceptible to brown rust, RSD, leaf scald. |
| Indonesia | Kenthung | Not available | Moderate germination ability; moderate stalk density; sporadically flowering; early-mid ripening variety; tolerant to top and steam borer; resistant to leaf scald, pokkah boeng, smut and mosaic; suitable for nonirrigated areas and regosol soil type with sufficient water resources. |
| Philippines | VMC84-524 | 16 | Intermediate to yellow spot; highly resistant to ring spot; very highly resistant to red rot of the midrib; moderately resistant to red rot of the leaf sheet; slight infestation of thrips; high tillering, fast growing, heavy trichomes. |
| | VMC86-550 | 11 | Susceptible to smot; susceptible to Downy Mildew; highly resistant to yellow spot; very highly susceptible to yellow leaf syndrome especially in the edge of field and waterlogged areas; highly resistant to rust; susceptible to borer. |
| | PHIL80-13 | 10 | Rated as sweet cane; low to medium tillering; versatile in varied soil and weather types; nontasselling. |

**Table 2.** Top varieties of sugarcane that cover between 30 and 50% of the area dedicated to this crop for major sugarcane producing countries.

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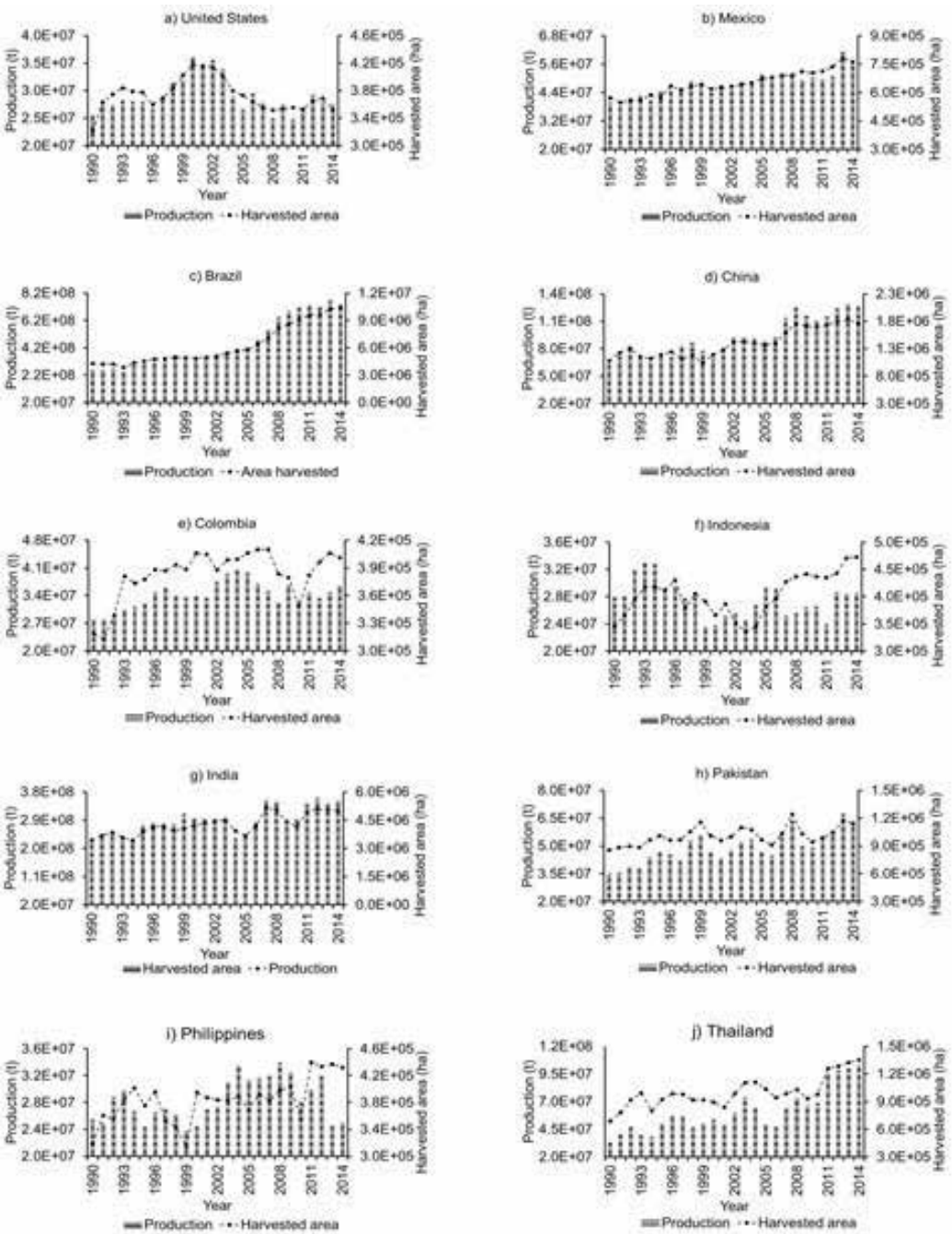


**Figure 2.** Annual production and harvested area for the period 1990–2014 in the principal sugarcane producers worldwide. United States (a), Mexico (b), Brazil (c), China (d), Colombia (e), Indonesia (f), India (g), Pakistan (h), Philippines (i) and Thailand (j). The data used to realize the charts were obtained from FAOSTAT [14].

The high- or low-correlation between the harvested area and sugarcane production can be an indicator of the good or bad treatment received by the soil in every planting period, depending on the infrastructure available to keep the soils healthy in each country.

The sugarcane varieties used in the countries dedicated to the production of this crop have special qualities to respond efficiently to the soil and weather characteristics of every place and have important properties of sucrose and biomass availability (**Table 2**). The International Society of Sugarcane Technologists collects and periodically publishes the most recent varieties of cane in each country dedicated to the sugarcane production.

The maturation period of these cane varieties is another important characteristic, which is taken into account in the moment to decide the harvesting period and the quantity of residue available to be burned. The two cane varieties of Brazil showed in **Table 2** [15] have the medium to late maturation (first variety) and early maturation (second variety), respectively; for China's varieties correspond to early maturation.

## 2. $CO_2$ emissions during the harvest of sugarcane in the major sugarcane producing countries

The database used to obtain and analyze the $CO_2$ emissions for the period 1990–2014 was acquired from FAOSTAT – Burning Crop Residues [16], whose empiric calculus depend of the Tier 1 methodology proposed by the Intergovernmental Panel on Climate Change. The main characteristics of these levels are the use of the basic information of every country, necessary to know a first estimation of the emissions of this greenhouse gas [17].

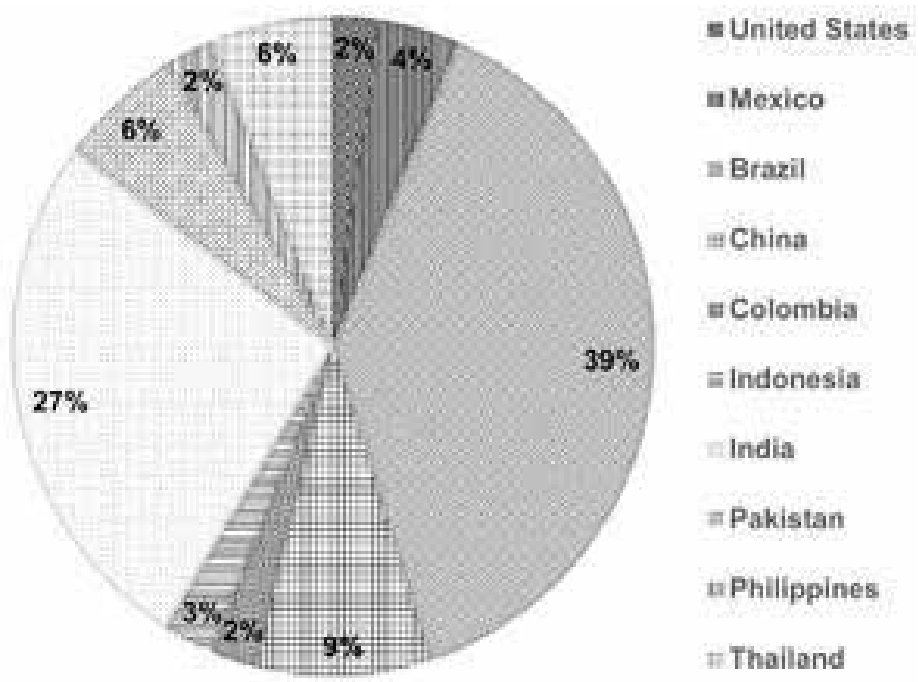


**Figure 3.** Total biomass burned during sugarcane harvest (dry matter).

The country with the major surface area dedicated to the sugarcane production is Brazil. Their total $CO_2$ emissions are generated in harvested areas by burning and green harvesting. In both cases, this greenhouse gas is emitted [18]. When sugarcane is burned, its variety planted plays an important role in the quantity of biomass available to be burned (**Figure 3**).

The average dry matter in the sugarcane ranks between 22.7 and 35.9% [19]. However, depending on the variety of sugarcane planted, it will have the real quantity of residue. For example, in Mexico, the main planted varieties have a residual fraction of 29%, from which 83% is dry matter [20].

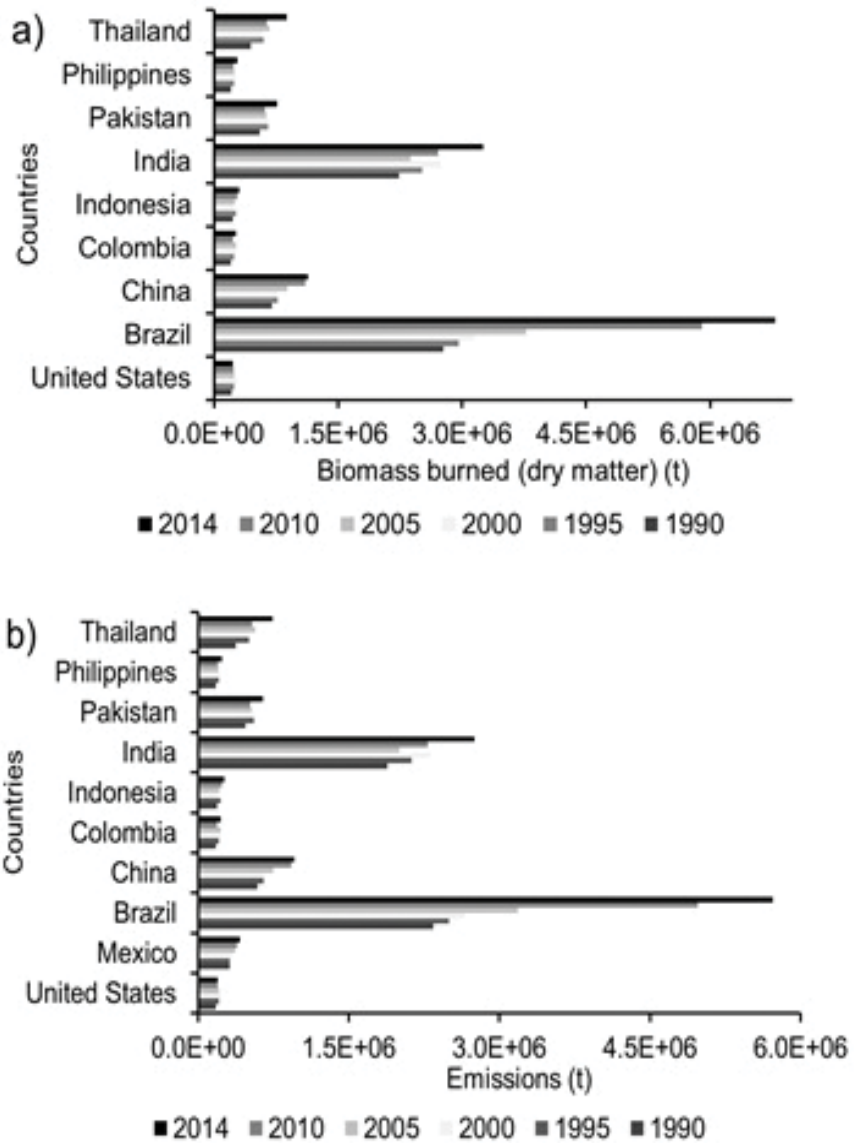


**Figure 4.** Biomass burned (dry matter) (a) and $CO_2$ emissions (b) by the major sugarcane producing countries.

There is a correlation between the biomass burned (**Figure 4a**) and $CO_2$ emissions (**Figure 4b**). Brazil, India and China have the highest level of both parameters. Their magnitude order is of millions of tons, and the magnitude order of sugarcane production by country (**Figure 2**) is higher than biomass burned because the dry matter is a percentage of the crop. In every year of the study period (1990–2014), the cane production, biomass burned and emissions of $CO_2$ have been increasing, not only by alimentary reasons but also by energetic needs reflected in the use of biomass to generate electric energy and the implementation of ethanol and its derivatives as fuels.

Actually there is no particular database in which the available nutrients for every planted period of sugarcane can be found. However, there exists general information that can help to understand the global distribution of soils and nutrients [21]. This information is important due to the volatilization of nutrients during the burning practice in every harvested period. The IPCC's methodologies estimate the greenhouse gas emissions, although other proposals, for example, the Seiler and Crutzen methodology [22], are very useful to know the amount of carbon and nitrogen released to the atmosphere during the burning of some crops.

A particular way to identify the conditions in which the sugarcane plantation was carried out is by referencing the values proposed to identify the "aptitude levels of sugarcane." It consists of identifying the soil and weather conditions in which the sugarcane cultivation takes place [23] and relates with the production per hectare (yield).

According to this methodology implemented for the sugarcane producing regions in Mexico during the period of 1990–2014, the yield per hectare ranked between 81.3 and 92.3 t $ha^{-1}$ [25]. This shows a high aptitude level for the country. It is not possible to use the same values from **Table 3** for other sugarcane cultivating countries because the edaphological and weather conditions are different.

| Property | High | Medium | Low | Not suitable |
|---|---|---|---|---|
| Annual temperature (°C) | 22–32 | 20/22–32/35 | 18–20 | <18 |
| Annual average precipitation (mm) | >1500 | 1250–1500 | 1250–1000 | <1000 |
| Solar radiation (h/year) | 1800–2200 | 1800–1400 | 1400–1200 | <1200 |
| Drought severity index | Absent | Slight | Strong to very strong | Severe |
| Slope (%) | 0–8 | 8–16 | 16–30 | >30 |
| Altitude (masl) | Up to 400 | 400–850 | 850–1300 | >1300 |
| Texture | Loam-Argillaceus | Argillaceus | Loam-sandy | Sandy |
| pH | 6.6–7.3 | 6.1–6.5, 7.4–8.3 | 5.6–6-0 > 8.3 | <5.5 |
| Organic matter (%) | >5 | 3–5 | 2–3 | 1–2 |
| Available nitrogen (kg/ha) | >300 | 300–225 | 225–150 | <150 |
| C/N relation | 8–12 | 12–15 | 15–30 | >30 |
| Expected yield (t $ha^{-1}$) | >80 | 55–80 | 40–55 | <40 |

**Table 3.** Aptitude levels of sugarcane. Proposed values for Mexico [24].

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## 3. Competitive use of the sugarcane waste

The sugarcane harvest can be done with or without burn. However, the ways to use the residues depend on the kind of processes involved during the cane lifting [26]. In general, the crop residues can be used as animal feeding and for energy generation. It is also used as a raw material for the production of honey, yeast, alcohol, hydrolyzed products, paper and fertilizers (**Figure 5**) [27].

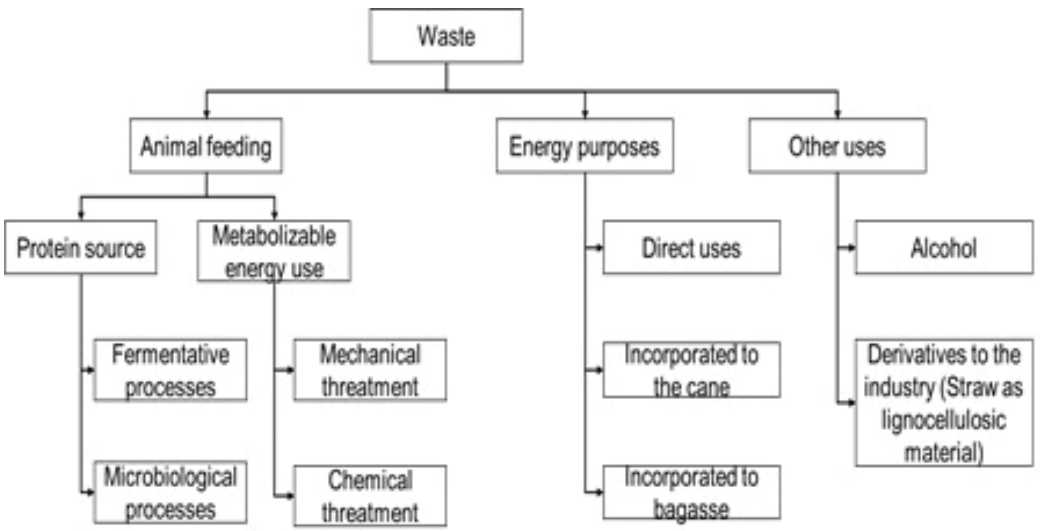


**Figure 5.** Uses of sugarcane straw [29].

| Country | Pearson correlation |
|---|---|
| United States | 0.54 |
| Mexico | 0.042 |
| Brazil | 0.74 |
| China | 0.40 |
| Colombia | 0.09 |
| Indonesia | −0.63 |
| India | 0.28 |
| Pakistan | 0.62 |
| Philippines | 0.44 |
| Thailand | 0.71 |

**Table 4.** Correlation between the harvested area and yield per hectare during the period 1990–2014.

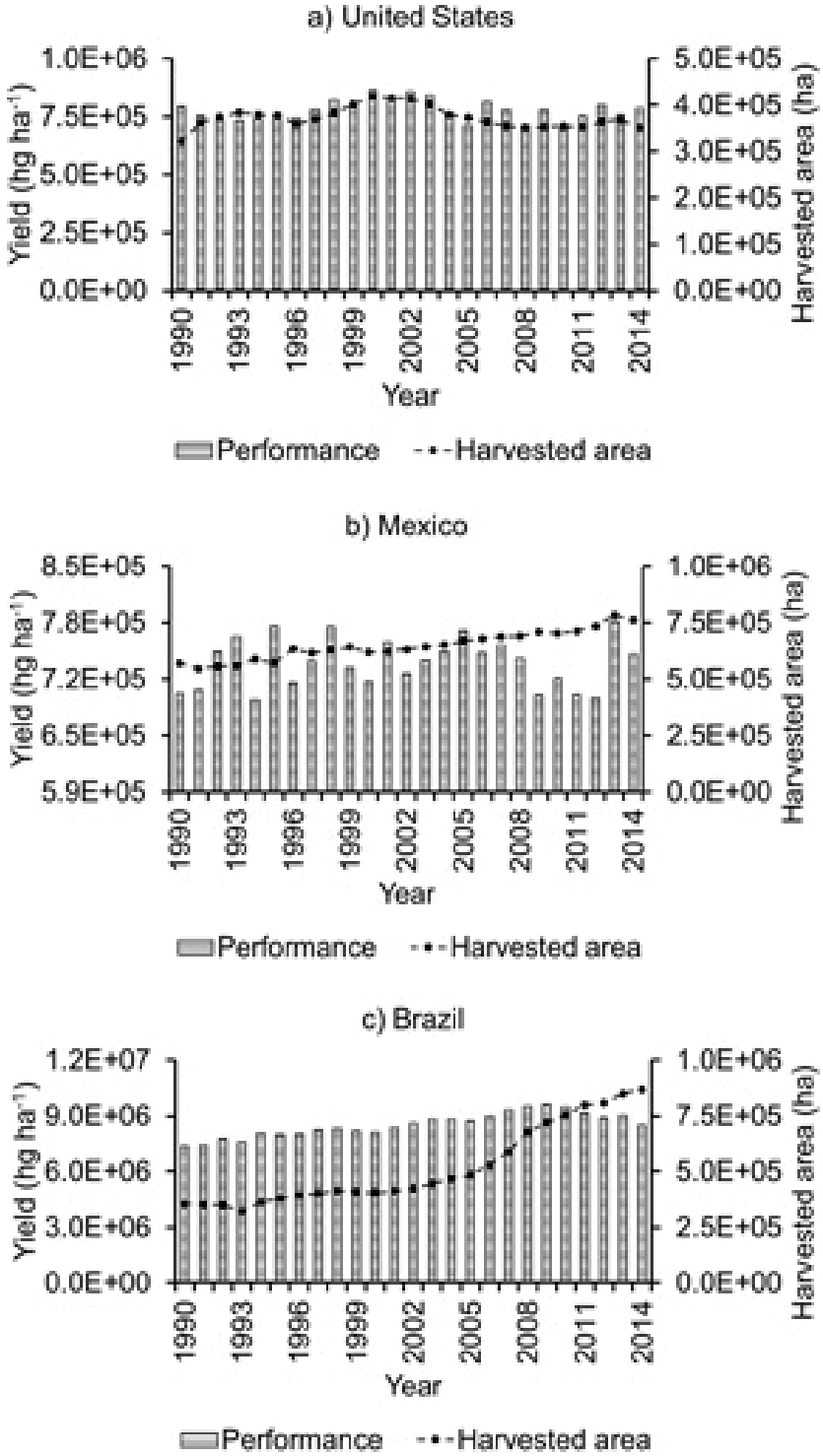


**Figure 6.** Relation between yield per hectare and harvested hectares in the United States (a), Mexico (b) and Brazil (c). 1 hg = 0.0001 t.

Actually, 85% of the world production of liquid biofuels corresponds to ethanol, where the main producers are Brazil and the United States because they contribute to 90% of their world production. The other 10% corresponds to Canada, China, European Union (France and Germany) and India (**Table 4**). The sugarcane plays an important role in the production of this fuel through fermentation and distillation processes [28].

The results shown above can be indicators of the capability of every country to take advantage of the crop residues to use it in an alternative manner. The Pearson correlation analysis between the harvested area and yield per hectare during the period 1900–2014 (**Table 4**) shows that countries such as Brazil and the United States have a better use of the planted soils, but for different reasons. For example, Brazil had a positive trend in both parameters during the studied years (**Figure 6c**), while the United States has increased the harvested hectares while keeping practically a constant yield per hectare.

Mexico had a positive trend in the harvested area, at a much higher level than the United States (**Figure 6a** and **b**) or the Philippines. However, the performance per hectare is constant but at lower levels than United States. This situation reveals that countries such as Mexico, Colombia and India have to invest more resources to keep their production levels constant and to generate useable residues for alternative uses.

The generation of waste from this crop in the sugarcane producing countries depends primarily on the performance of sugarcane production. In this context, the major biofuel (ethanol and biodiesel) producing countries are Brazil, the United States, China and India [30].

## 4. Conclusions

The biomass (dry matter) available to be burned during the harvest period of sugarcane, play an important role in the $CO_2$ emission levels generated by the countries that practice this activity, and its release into the atmosphere can increase or decrease due to other factors such as soil quality, cane varieties used and weather conditions.

In this chapter, we can see the important relation between the production levels and harvested areas, an extensive harvest surface not necessarily give high production levels. Countries such as Colombia, Indonesia and Philippines had this behavior in their planted soils in different years. On the other hand, Brazil, China, India, Mexico, the United States, Pakistan and Thailand had a good correlation between both the parameters because when the harvested hectares increased or decreased, the production levels remain the same.

During the study period (1990–2014), we can see that Brazil, India and China had the highest quantity of cane waste (dry matter) burned and simultaneously had the better production levels and the major emissions of $CO_2$. In general, the countries analyzed had a positive trend reflected in the annual increase of its emissions, except for the United States which reduced its production levels since 2004.

The countries that kept a good correlation between their yield levels and harvested area during the study period, it is because they have had the infrastructure to prepare their soils adequately and use the cane varieties that can be adapted to each condition presented in every

stage of growth in the best way, but also have major possibilities to take advantage of the available cane waste and give it an alternative use.

Finally, to reduce $CO_2$ emissions, it is not necessary to reduce the production levels, rather, good performance must be maintained using appropriate planting and harvesting techniques which also allow the waste (dry matter) to be disposed of in suitable conditions to be used. Actually, it could be expensive to implement these alternative practices, so every country must generate a new mechanism to make it more feasible.

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Section 3

# Propagation and Biotechnology

Chapter 6

# *In Vitro* Propagation of Sugarcane for Certified Seed Production

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Additional information is available at the end of the chapter

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**Abstract**

Micropropagation of sugarcane is important to obtain pathogen-free plants, genetically homogeneous and invigorate. The micropropagation procedure is divided into stages for the sake of better understanding. Micropropagation for large-scale sugarcane production using a temporary immersion system (TIS) is described. In addition, the aim of this chapter is to report, from the laboratory to the field, the best way to establish and use basic seed (primary seed), semicommercial seed (foundation or secondary seed) and commercial seed production. In conclusion, commercial sugarcane micropropagation enables the massive multiplication of plants to obtain certified vitroplants and increase the sugarcane and sugar productivity per unit area.

**Keywords:** biotechnology, micropropagation, temporary immersion system, vitroplants, seed production

## 1. Introduction

Sugarcane cultivars (*Saccharum* spp. hybrids) are grown mainly for sugar, ethanol and subproducts. Its large-scale production makes it an important crop in the tropical and subtropical regions of many countries [1]. Despite the importance of sugarcane cultivation, its production is generally characterized by low yield in the field due to, among other causes, virtually zero renewal of plantations because of the lack of vegetative materials certified free of pests and diseases. According to Flores [2] and Lal et al. [3], sugarcane varieties age over the years, losing their productive power, which can deteriorate and eventually disappear from the commercial crop. In many countries, most vegetative seed is propagated by conventional methods

by sowing bud-containing cuttings. However, this technique does not ensure the sanitation and rejuvenation of the selected varieties in the field. An alternative to this problem is the use of Plant Tissue Culture (PTC) techniques [4]; this plant biotechnology tool allows the establishment, manipulation and development, under artificial and controlled conditions, of cells, tissues or organs and is very useful for the regeneration of rejuvenated, genetically homogeneous plants free of pests and diseases.

In sugarcane, *in vitro* propagation or cloning of plants uses PTC techniques to obtain a constant supply of plant material, unlike conventional vegetative propagation, which is seasonal in nature. Sugarcane micropropagation has allowed the rapid multiplication of new varieties, rejuvenation of old deteriorated varieties and sanitation of diseased varieties [3] and has also facilitated the exchange of in vitro plant material. Currently, semiautomation of micropropagation by temporary immersion systems (TISs) offers a practical strategy to reduce production costs [5]. TISs are semiautomated bioreactors designed for the mass propagation of cells, tissues, embryos or organs using liquid medium [6]. TISs have been shown to be a powerful tool for sugarcane propagation [7].

A micropropagation-based crop has prominently better quality than a conventionally raised one. According to Sawant et al. [1], sugarcane micropropagation increases productivity in the field by up to 25%, while Pérez et al. [8] mention that the combined effect of in vitro sanitation and rejuvenation is expected to increase sugar yields by between 10 and 15% per unit area. These advantages have allowed the commercial exploitation of micropropagation in the sugar industry worldwide. This technology is now used to supplement commercial sugarcane production in many countries including Brazil, India, the USA and Cuba [4].

Certified vitroplants obtained from in vitro propagation systems are used in the field for the production of quality seed. This technology helps farmers to enhance crop productivity. The aim of this chapter is to report, from the laboratory to the field, a seed production system comprising basic seed (primary seed), foundation (secondary seed) and commercial seed production to obtain a commercial crop.

## 2. *In vitro* propagation

Sugarcane micropropagation enables the identical production of the selected cultivars using PTC techniques. PTC refers to growing and differentiation of cells, tissues and organs isolated from the mother plant, on artificial semisolid or liquid media under aseptic and controlled conditions. The small organs or pieces of tissue used in PTC are called explants. PTC medium provides inorganic nutrients and usually a carbohydrate to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis. When carbon is supplied with sucrose and kept in low light conditions, micropropagated plantlets are not fully dependent on their own photosynthesis.

To date, sugarcane micropropagation has shown great productive potential [3]; it is being used in commercial laboratories to obtain certified plant material.

The sugarcane micropropagation process is carried out in the following stages [9].

**Stage 0: mother plant selection.** Donor cultivars are selected and conditioned to be used to initiate in vitro cultures. It should be considered that the mother plant corresponds to the selected variety; in many cases, there is a varietal mixture in commercial cane plantations. The genetic purity of the variety should be certified by the breeder or research organization identified for the maintenance of the variety.

**Stage I: in vitro establishing.** The choice of the apical meristems (explants) and their disinfection is carried out to initiate an aseptic in vitro culture. Apical meristem culture produces virus-free sugarcane plants. The meristem remains in an active state during the vegetative growth phase, and the meristem cells are in a permanent totipotent state.

**Stage II: multiplication.** It is at this stage that mass propagation is performed, obtaining many new shoots from minimal amounts of tissue. Based on our experience, we recommend making no more than eight subcultures because above that level the length and number of shoots decrease. In addition, the likelihood of genetic variants occurring increases.

**Stage III: elongation and rooting.** The shoots must form their root system and at the same time increase their size to facilitate their manipulation and adaptation to the acclimatization conditions.

**Stage IV: acclimatization.** This process is carried out in a greenhouse. It consists of a slow reduction of the relative humidity and gradual increases in the luminous intensity for a better adaptation to the external environment. The greenhouse infrastructure must ensure control of both relative humidity and light entry.

It is important to mention that the elongation and rooting stage varies according to the method being utilized; it is not always necessary when semiautomation of micropropagation by TISs is used.

Conventional micropropagation of sugarcane in semisolid media has been reported [10, 11]. However, to reduce the labor required and increase efficiency, temporary immersion systems (TISs) have been successfully used to improve in vitro sugarcane multiplication [12–16]. The principle of these systems is the immersion of explants for a determined time and frequency.

We have implemented different TISs for commercial sugarcane micropropagation. Sugarcane meristems (cv. Mex 69–290) were collected from field-grown plants and cultured following the protocol of Jiménez et al. [17] The 3-cm-long sugarcane shoots after three subcultures (30 d each) were used as explant. Explants (two shoots each) were placed in the Temporary Immersion Bioreactor (TIB™, Cuba), the Recipient for Automated Temporary Immersion (RITA™, France), the Gravity Immersion Bioreactor (GIB, Mexico) and the SETIS™ Bioreactor (Belgium) containing MS [18] medium supplemented with 30 g/L sucrose, 1 mg/L Kinetin (Sigma Chemical Company, MO, USA), 0.6 mg/L 3-indoleacetic acid (IAA, Sigma Chemical Company, MO, USA) and 0.3 mg/L 6-benzylaminopurine (BAP, Sigma Chemical Company, MO, USA). The pH of the culture medium was adjusted to 5.8 with 0.1 N sodium hydroxide and then autoclaved at 1.2 kg/cm$^2$ for 15 min at 120°C. Three replicates were used in all experiments. TISs were incubated at 24 ± 2°C and were maintained under fluorescent light (40–50 μmol m$^{-2}$ s$^{-1}$) and a photoperiod of 16 h. Immersion frequency was according to Lorenzo et al. [12] After 30 d of incubation, the number and length of shoots per explant were assessed.

A completely randomized experimental design was used for all experiments. Results were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's comparison of means test ($p \leq 0.05$) using SPSS statistical software (version 22 for Windows).

When evaluating the different TISs in sugarcane during in vitro propagation, significant statistical differences were observed for the number and length of shoots per explant. The bioreactors with the highest number of shoots per explant were TIB, GIB and SETIS, with 38, 40 and 41 shoots/explant, respectively, followed by RITA, with 32 shoots/explant. Regarding shoot length, the bioreactors with the longest length were TIB, GIB and SETIS with 8.6, 10.7 and 9.8 cm in length, followed by RITA with 6.0 cm in length (**Table 1**).

Semiautomation of sugarcane micropropagation using TISs is a strategy to reduce production costs. The TIB, GIB and SETIS bioreactors showed good performance in the formation of the length and number of shoots; probably their size, among other factors, favors the development of explants. On the other hand, RITA, due to its limited capacity, did not allow an increase in length and number of new shoots. Commercial sugarcane micropropagation by TISs is shown in **Figure 1**.

| TIS | No. of shoots/explants | Shoot length (cm) |
|---|---|---|
| RITA | 32.8 ± 0.58 b | 6.0 ± 0.27 b |
| TIB | 38.8 ± 0.67 a | 8.6 ± 0.43 a |
| GIB | 40.0 ± 0.55 a | 10.7 ± 0.33 a |
| SETIS | 41.0 ± 0.40 a | 9.8 ± 0.47 a |

Values represent mean ± SE (standard error). Means with different letters per column represent statistical difference (Tukey, $p \leq 0.05$).

**Table 1.** Sugarcane (*Saccharum* spp. hybrid cv. Mex 69-290 micropropagation by different temporary immersion systems (TISs)).

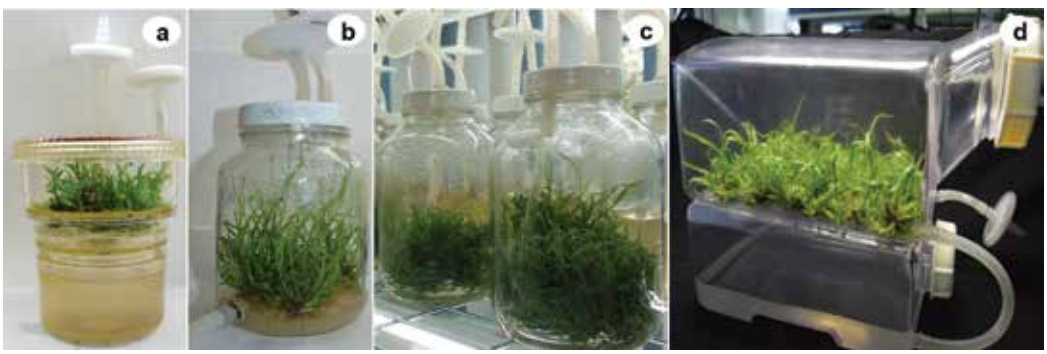


**Figure 1.** Sugarcane micropropagation by temporary immersion systems. (a) Recipient for automated temporary immersion (RITA™), (b) gravity immersion bioreactor (GIB), (c) Temporary Immersion Bioreactor (TIB) and (d) SETIS™ bioreactor, after 30 d of incubation.

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## 3. Genetic homogeneity

The genetic or epigenetic variation obtained by different in vitro propagation systems is called somaclonal variation [19]; it is a problem that affects commercial micropropagation, where it is necessary to maintain the maximum genetic homogeneity of the regenerated individuals with respect to the mother plant.

The causes of somaclonal variation are not well understood and have not been fully elucidated [20]. However, some factors that determine the frequency of somaclonal variation include the in vitro regeneration system, the type and concentration of growth regulators applied, and the number of subcultures [21]. Consequently, it is important to determine the optimal number of subcultures that can be made from an explant for each sugar cultivar to be micropropagated.

Martínez-Estrada et al. [22] determined by inter-simple sequence repeat (ISSR) markers that no more than eight subcultures should be done due to the existence of polymorphism between the subcultures produced by a Temporary Immersion Bioreactor (**Figure 2**), since above eight subcultures the length and number of shoots decrease.

Genetic homogeneity and plant health are two important quality aspects that must be addressed before the seedlings are distributed outside the laboratory. According to Lal et al. [3], contamination of cultures is a severe problem that not only reduces the frequency of shoot culture initiation from the source explants but also the total number of shoots produced at various cycles of cultures. Plant tissues could also be cultured in the presence of bacterial and/or fungal contaminants. Therefore, a phytosanitary diagnosis should be required.

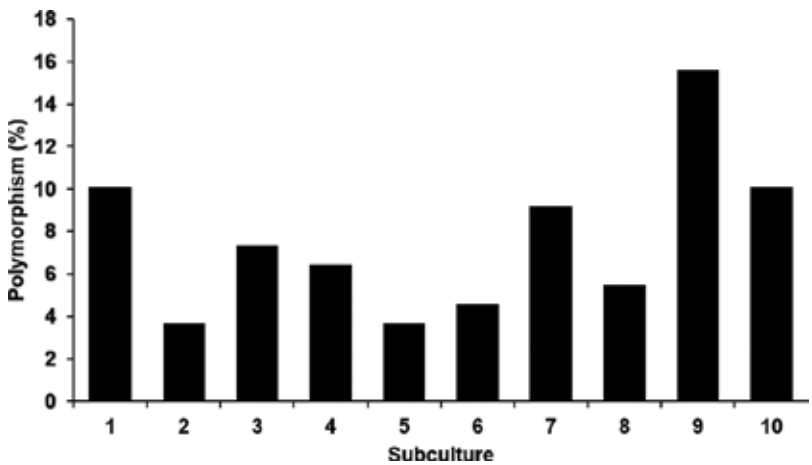


**Figure 2.** Effect of subculturing on polymorphism percentage of shoots of sugarcane (cv. Mex 69–290) using Temporary Immersion Bioreactors assessed by inter-simple sequence repeat (ISSR) markers. Each bar represents the polymorphic percentage of subcultures 1–10.

## 4. Phytosanitary diagnosis

Diseases represent one of the main factors that affect sugarcane production. Knowledge of phytosanitary status and the correct identification of phytopathogens are key to reducing losses due to diseases. In this regard, it is essential to carry out a phytosanitary diagnosis at an early stage to ensure the phytosanitary quality of the seedlings obtained in the laboratory.

In many countries where sugarcane is an important part of the economy, plant health departments (PHDs) are responsible for certifying the procedures for obtaining pest- and disease-free sugarcane vitroplants. The PHD determines the requirements necessary for accreditation of micropropagation laboratories engaged in in vitro culture of sugarcane, whose legal basis is determined based on Plant Health Laws. To obtain certification, a laboratory must meet a series of requirements that demonstrate technical competence, satisfactory infrastructure and sanitary capacity to produce plant material in vitro.

Accreditation of micropropagation laboratory: micropropagation laboratory should be accredited by an appropriate authority to ensure technical competence and satisfactory infrastructure.

| **Common name** | **Scientific name** |
|---|---|
| Sugarcane scale | *Aulascaspis tegalensis* |
| Sugarcane leafhopper | *Perkinsiella sacharicida* |
| Sugarcane leafhopper | *Pyrilla perpusilla* |
| Spotted stalk borer | *Chilo partellus* |
| Sugarcane borer | *Eldana saccharina* |
| Purple stem borer moth | *Sesamia inferens* |
| Kenya mealybug | *Planococcus kenyae* |
| Giant moth borer | *Castnia licoides* |
| Sugarcane downy mildew | *Peronosclerospora sacchari* |
| Sugarcane gumming disease | *Xanthomonas campestris pv. vasculorum* |
| Ratoon stunting disease | *Leifsonia xyli* ssp. *xyli* |
| Bacterial canker | *Dickeya chrysanthemi* |
| Bacterial wilt | *Pantoea stewartii* |
| Leaf scald of sugarcane | *Xanthomonas albilineans* |
| Virosis | *Sugarcane Streak Virus* |
| Virosis | *Sugarcane Sereh virus* |
| Sugarcane smut | *Sphacelotheca erianthi* |
| Sugarcane smut | *Sphacelotheca macrospora* |
| Sugarcane smut | *Ustilago scitaminea* |

**Table 2.** Pests and diseases which plant material produced *in vitro* must be free of.

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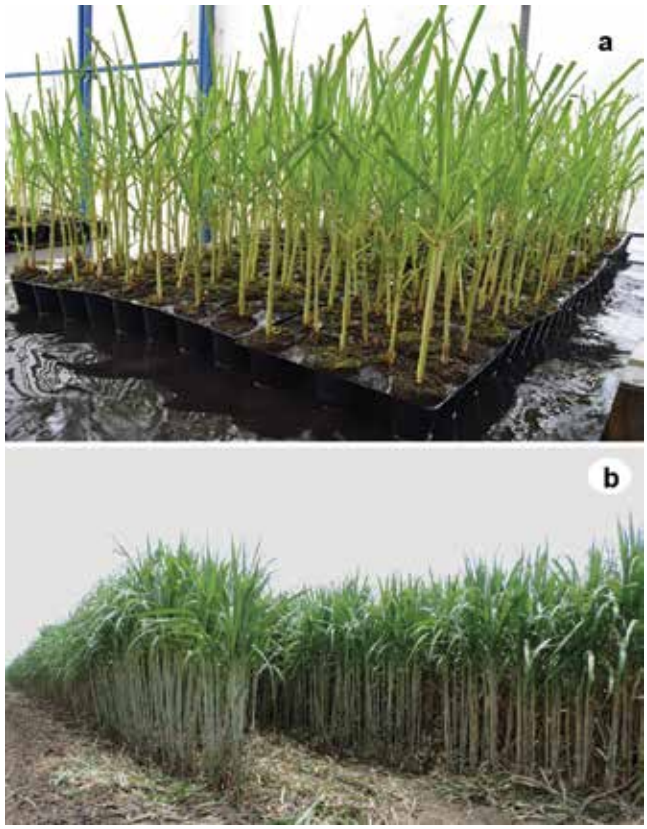


**Figure 3.** Certified vitroplants obtained from laboratory. (a) Vitroplants ready for field transfer and (b) field planting.

To determine plant health, samples of sugarcane shoots are taken *in vitro* and sent to the Phytosanitary Diagnostic Centers authorized to verify the plant health. *In vitro* propagated plants should be indexed for freedom from viruses and virus-like diseases through enzyme-linked immunosorbent assay (ELISA) and molecular methods [23].

According to the phytosanitary diagnosis report and based on the Plant Health Law, the diagnosis must be negative for the main sugarcane diseases (**Table 2**).

After obtaining healthy in vitro plants, a certification is issued so that vitroplants can be used for the establishment of certified basic seed nurseries (**Figure 3**).

## 5. Seed production

Seed production system comprises basic seed (primary seed), semicommercial seed (foundation or secondary seed) and commercial seed production.

The unit area of the seed nursery should be approximately one-tenth of the area that is planned to be renewed each year in the commercial plantations. This plantation system is used because

the multiplication rate is around 1:10 (10 internodes/stem). Sugarcane stem used for seed production is composed of a series of internodes; each internode forms a new plant. Harvesting of each seed nursery takes place after 7–10 months of development, when the plants have the required physiological conditions to obtain seed (internodes). Physiological maturity also depends on the type of variety used. After the harvest, heat treatment of internodes helps to get rid of several diseases and pests. According to Jalaja et al. [23], for seed heat treatment, thermohydrotherapy is recommended. Internodes are immersed in water maintained at 50°C for 2–2.5 h. Fungicides and bactericides are mixed in hot water to eliminate diseases. Proper thermohydrotherapy and pesticide application ensure the eradication of diseases and insect pests. Each seed nursery is described below.

The basic seed nursery is established with vitroplants. The health status of the seeds should be adjusted to those of each country. In seed nurseries, two phytosanitary assessments should be carried out to detect off-types (mutations or varietal mixture) and to remove plants infected with diseases or pests: the first, at 4 months of age, and the second, immediately before the seed cut, at which time stem samples are randomly taken. With approximately 10,000 vitroplants/ha, with an average distance between furrow and plant of 1 × 1 m, it is possible to establish basic seed nurseries. However, the sowing density depends on the type of variety and cropping system.

The *semicommercial seed* nursery is planted with material from the basic seed or with material from the ratoon of another semicommercial seed nursery that has been heat treated. The area of this field is, in general, 10 times greater than that of the basic seed nursery; as in this one, at 4 months of age, a phytosanitary evaluation must be done, and at the time of the cut, samples must be taken for phytosanitary diagnosis.

The *commercial seed* nursery is established with material from the template or the first ratoon of a semicommercial seed nursery. The area is at least 10 times greater than that of the semicommercial seed nursery. Although in this case it is not necessary to thermally treat the material, it is recommended and the same phytosanitary evaluations should be carried out. This seed nursery must have a sanitary state like that presented by the semicommercial seed nursery.

In the seed production system, it is important that the growing area has some type of irrigation so that the seedlings do not suffer from stress. Seed has to have a high water content and good nutritional status. In addition, plants must receive all the care and practices required for good development. In order to ensure the proper development of each seed nursery, a technological package is required that includes crop fertilization and adequate pest, disease and weed control.

In each seed nursery, the following criteria must be met:

- *Plant health*: plants free of pests and diseases.
- *Genetic purity of material*: high varietal purity, there being no more than one variety within the seed nursery (varietal mixture).
- *Genetic homogeneity*: clonal fidelity, identical plants without somaclonal variants (mutations).

Finally, the commercial plantation comes from the template or ratoon of a certified commercial seed nursery. This area is 10 times greater than that of the commercial seed. Commercial

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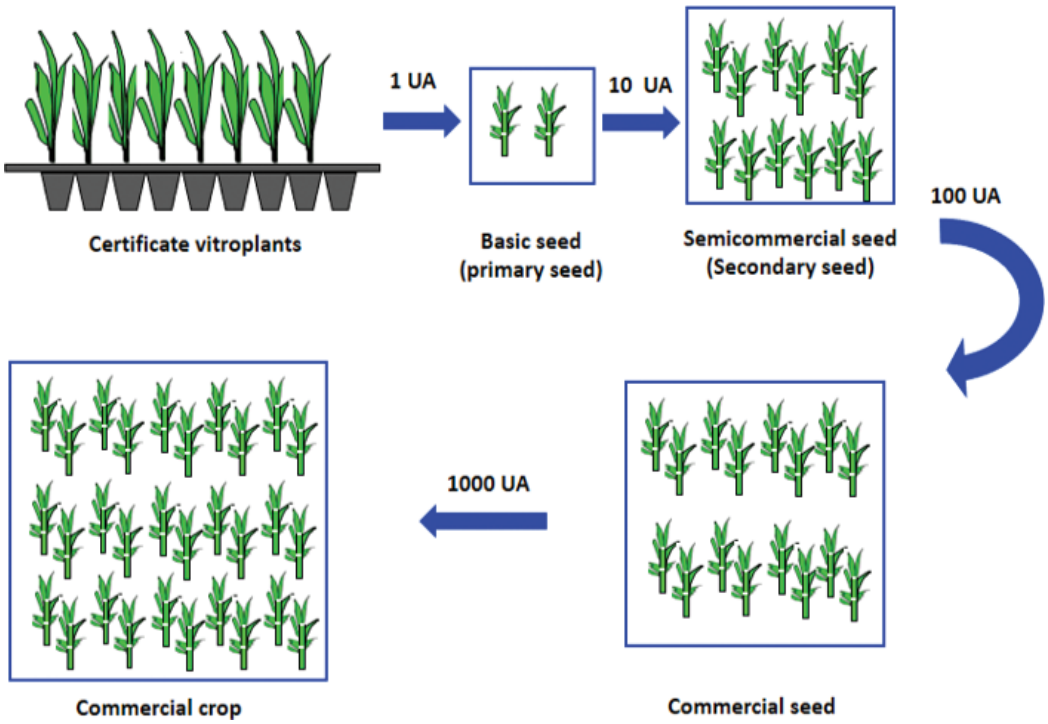


**Figure 4.** Seed production system comprising basic seed (primary seed), foundation (secondary seed) and commercial seed production to obtain a commercial crop per unit area (UA).

seed should preferably be located near the commercial crop to minimize transportation costs and damage during transport. The sowing time of the commercial crop determines the time for establishing the seed nursery. This is because there are early, intermediate and late crop-cycle cultivars; other factors include the availability of irrigation or whether it is subject to a seasonal sowing calendar. The fields for this purpose must be chosen from among the best according to the physical and chemical conditions of the soil and water availability. For the commercial crop, a change of seed is required once every 4 or 5 years. This change of seed favors the repopulation of the plantation, as well the rejuvenation and health of the crop, which recovers its productive potential. It is important to mention that canes from the ratoon crop, however, should not be used for seed production [23]. This is because ratoon cropping involves growing a fresh crop from the suckers of the plant crop without replanting (second crop). Therefore, a decline in cane yield in successive ratoon crops has been reported; the causes for this decline are poor ratoon management, inherited differences in potential productivity and an increasing incidence of diseases which results in a gappy stand [24]. **Figure 4** summarizes the procedure for the seed production system to obtain a commercial crop.

In conclusion, commercial sugarcane micropropagation enables the massive multiplication of plants to obtain certified vitroplants and increase the sugarcane and sugar production per unit area. This technology helps farmers to enhance their crop productivity. In addition, a sugarcane micropropagation laboratory can be used for in vitro conservation of germplasm,

application of biotechnology for genetic improvement programs and easy transportation during exchange of in vitro plant material between countries.

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Chapter 7

# Biotechnological Interventions for the Improvement of Sugarcane Crop and Sugar Production

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Additional information is available at the end of the chapter

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**Abstract**

Sugarcane, not only fulfills 70% of world sugar needs but is also a prime potential source of bioethanol. It is majorly grown in tropical and subtropical regions. Researchers have improved this grass to great extent and have developed energy cane with ability to accumulate up to 18% sucrose in its Culm. Improvement of this crop is impeded by its complex genome, low fertility, long production cycle and susceptibility to various biotic and abiotic stresses. Biotechnological interventions hold great promise to address these impediments paving way to get improved sugarcane crop. Further, being vegetatively propagated in most of the agroecological regions, it has become more attractive plant to work with. This chapter highlights, how advanced knowledge of omics (genomics, transcriptomics, proteomics and metabolomics) can be employed to improve sugarcane crop. In addition, potential role of *in vitro* techniques and transgenic technology has also been discussed for developing improved sugarcane clones with enhanced sugar recovery.

**Keywords:** sugarcane, omics, transgenics, crop improvement, biofuel

## 1. Introduction

Sugarcane is a major contributor to world sugar and more than 70% of global saccharine needs are fulfilled by this sweet grass. It has been cultivated since pre-historic times as a sugar source. Further, it has great potential to be used for the production of bioethanol and has been grown in many countries as an energy crop. Brazil is the top most consumer of sugarcane biofuel followed by USA and fulfill 50% of their energy needs through biofuel. They run more than 5.0 million automobiles on hydrous ethanol at an ethanol content of 95.5% [1]. These facts direct us to strive for the improvement of sugarcane crop so that global energy needs may be fulfilled

sustainably. Various promising varieties have been developed so far but they are posed to certain drastic stresses including biotic as well as abiotic stresses. Similarly, efforts have been made to improve sugar recovery. Since, crop productivity and quality can only be improved by employing innovative technologies. Plant tissue culture and genetic engineering has great potential to resolve problems faced by this crop [2]. Transgenic technology can do a lot to address all the aforementioned yield limiting constraints as any of the alien genes may be introduced into the plant through genetic transformation methodologies. Different methods of transformation i.e. biolistic [3, 4] *Agrobacterium* [5] and electroporation [6] have been employed to engineer valuable agronomic traits like resistance against weedicides [7], viruses [8] and insects [3]. Efforts have been made to engineer metabolic pathways for improved sugar content [9] and for the production of biopolymers and bioplastics. Omics approaches have contributed a lot to understand and explore sugarcane genome to develop improved clones. Milestones in structural and functional genomics are also convincing. Different types of markers have been developed to speed up molecular breeding through early identification of superior genotypes [10]. Thus, biotechnological interventions have great potential to promote sugarcane not only as future energy crop but also a factory house for the production of therapeutics and industrial compounds.

These interventions have been discussed here to focus critical areas of research that can attract researchers for the improvement of this crop. Understanding molecular mechanisms involved

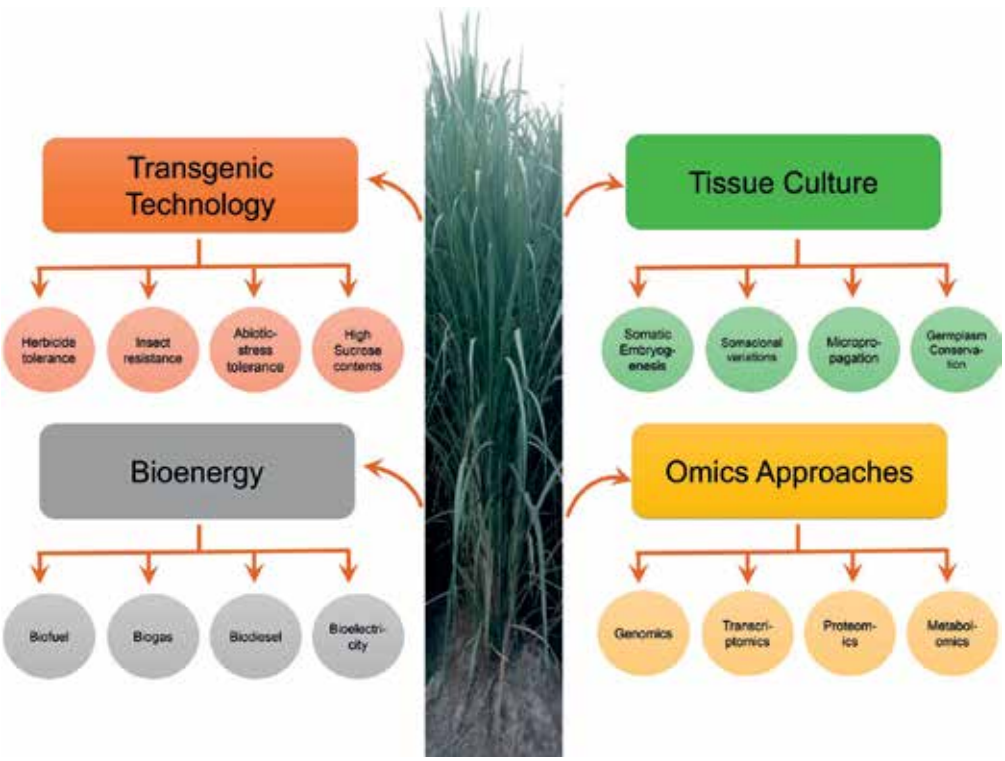


**Figure 1.** Schematic sketch showing role of biotechnological interventions for the improvement of sugarcane crop and sugar production.

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in metabolic pathways and sucrose accumulation will prove a real milestone in developing future energy crop. Similarly, importance of *in vitro* techniques have been highlighted that how advancements in tissue culture techniques are important for germplasm conservation, development of somaclonal variants and genetic transformation. Likewise, potential of transgenic technology has been discussed to develop insect resistant, disease resistant and herbicide tolerant plants. Omics, a real potential area of future research, has been overviewed to highlight the role of genomics, transcriptomics, proteomics and metabolomics in sugarcane crop improvement and in developing energy cane (**Figure 1**).

## 2. Tissue culture based approaches for sugarcane crop improvement

Since the pioneer work on callus induction at Hawaiian Sugar Planters' Association Experiment Station and the method developed by Nickell [11] for root production, sugarcane tissue culture appeared as a valuable tool for diverse research activities. Shortly after this, Heinz and Mee [12] published the first report on sugarcane regeneration. These *in vitro* techniques had huge impact on basic research and also on the research of commercial interest which includes maintenance of elite germplasm, production of agronomically superior somaclones, micropropagation of elite clones, healthy planting material and screening for abiotic/biotic stress tolerance.

### 2.1. Somatic embryogenesis

Somatic embryogenesis may be divided into two phases: induction and expression. During the induction phase, embryogenic competence is acquired by differentiated somatic cells whereas during expression phase, embryogenic cells differentiate into somatic embryos. Komamine et al. [13] evaluated that embryogenic cells did not require any exogenous stimuli in the form of growth hormones or vitamins at induction state. Rather, competent cells require exogenous stimuli at transitional state in very minute quantities. Somatic embryogenesis may either be direct or indirect. Direct somatic embryogenesis involves development of embryo directly on the surface of explant tissues i.e. stem segments, leaf segments, young inflorescence, zygotic embryo, protoplasts and microspores. Indirect somatic embryogenesis involves an intermediary step of callus induction followed by embryogenesis. Different factors have been found to play key role in the acquisition of embryogenic competence. Exogenous growth regulators promote embryogenic competence by affecting cell polarity, pH gradients and by modifying ionic balance all around the cells. Intracellular pH is very crucial for embryogenic competence acquisition. Another critical aspect is the physical isolation of a cell from others. Embryogenic competence acquisition by somatic cells is regulated by the expression of certain genes which involve either upregulation or down regulation of certain functional genes. In addition, physiological, morphological and metabolic variations are also very important for the acquisition of embryogenic competence. Thus, somatic embryogenesis is a great milestone in sugarcane biotechnology [14]. Originally this method was developed as a substitute of meristem culture and regeneration but now it has become an important component of genetic engineering as well. It has well been exploited for the preservation of mutants and transformed material till

their approval or field establishment. Various high yielding, early maturing, high sucrose content and smut resistant varieties with good ratooning ability have also been developed through somatic embryogenesis.

### 2.2. Somaclonal variations

Somaclonal variation have been employed to improve cane-quality, sugar recovery, yield, drought tolerance and disease resistance. To increase the incidence of genetic variation and to get positive modifications in the target plant genome, physical (ion beams, gamma rays) and chemical (sodium azide, sodium nitrite and ethylmethane sulfonate (EMS) mutagens have been tested [15]. Introduction of selection pressure at cellular level has been successful to isolate mutants with desired characters. For fungal pathogen resistance inoculation with fungal pathotoxins or culture filtrates has been very effective. Somaclonal variants of sugarcane were developed against eye spot disease by Larkin and Scowcroft. Mutagenesis has been used by various researchers to isolate embryogenic cells and plants tolerant against red rot [16].

Similarly, for abiotic stress tolerance polyethylene glycol, mannitol and sodium chloride has been used for the selection of plants against drought/salinity tolerance [17]. Various studies were conducted to evaluate the level of variability and transmission of variations into the next generation by vegetative propagation. These studies verified the occurrence of considerable variations in *in-vitro* derived plants. However, extensive field experiments showed that tissue culture derived phenotypic variations were often temporary as most of the variants relapsed to the parental phenotype in the first ratoon crop [18]. Few other studies also supported somaclonal variations but to little extent. Chowdhury and Vasil [19] were not able to recognize any considerable variation in the DNA of plants regenerated from cell suspension, protoplasts and callus cultures. Taylor et al. [20] performed random amplified polymorphic DNA (RAPD) analyses of plants regenerated from embryogenic culture. Only a few polymorphisms were observed. Anyhow, there are different opinions about the somaclonal variations and their transmission to next generation. Most of the researchers are of the view that these variations depend upon the genetic makeup and experimental conditions under which plants are screened and selected.

### 2.3. Micropropagation to produce sanitated plants

Systemic buildup of infections particularly diseases (viral, bacterial and fungal) affect plant vigor and health. Unavailability of an efficient *in vitro* mass multiplication system in sugarcane is a major constraint in the provision of disease free elite germplasm [21]. Sugarcane has long breeding cycle and it requires 10–15 years to complete selection cycle. Fuzz multiplication rate of newly released sugarcane varieties is always slow 1:6–1:8 [22] and diseases accumulate in the seed sets during multiplication. Unavailability of disease free planting material is a major limitation in the improvement of sugarcane crop. Normally, sugarcane reproduces vegetatively but seed propagation is also there under particular climatic conditions [23]. Nodal cuttings are being used for the propagation of commercial sugarcane. In this method of multiplication, meristematic or non-meristematic tissues are used as explant. Sugarcane plants have been regenerated directly from apical and axillary meristems and also from immature leaf tissues [24].

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*In vitro* propagation of sugarcane through meristematic tissues responded better as compared with other types of plant tissues. Therefore, significant efforts have been made to explore meristematic tissues for mass multiplication. In the beginning of twenty-first century, some reports highlighted direct regeneration of sugarcane genotypes through thin layer culture of cells from immature leaf or inflorescence [24]. They reported to lessen the time span required for *in vitro* propagation. Significant efforts have been made to establish protocols for direct or indirect sugarcane regeneration. Almost each part of the sugarcane plant has been exploited for callus induction but only inflorescence and immature leaves [25] responded better to morphogenic callus. Callus based regeneration gained significance with the prediction that *in-vitro* induced mutations can play some key role in sugarcane improvement [26]. *In vitro* induced variability is beneficial for the development of new varieties but it becomes undesirable when true-to-type plants are required. Only fewer examples have been quoted to highlight useful variations in callus-derived plants. Meristem culture was successfully used to eradicate chlorotic streak disease, sugarcane mosaic virus [27], white leaf disease and ratoon stunting disease. Combination of meristem culture and heat treatment have proved very effective to eradicate pathogens of Fiji disease [28], SCMV [27] and downy mildew. It is an effective method to eliminate most of the bacterial and fungal diseases and is commonly used to eradicate diseases of unknown etiology as well. Disease free planting material of sugarcane obtained from apices culture is now routinely used for the international exchange of this crop. Researchers have explored that plants regenerated from thin cell layer culture can be used to produce disease free sugarcane plants from the infected ones with *Leifsonia xyli*, SCMV and FDV. *In vitro* culture techniques are used in Brazil and USA to produce healthy planting material for commercial applications. Cryotherapy has also appeared as a proficient method to eliminate phytoplasma from the crop plants and has also been used for long term storage of germplasm or production of disease free plants [29].

### 2.4. Germplasm conservation

Another important application of *in vitro* techniques that attracted researchers is germplasm conservation [30]. *In-vitro* storage of sugarcane germplasm had been established at the Centre de Cooperation Internationale en Recherche Agronomique pour le Development (CIRAD) in France, Sugarcane Breeding Institute in India, and BSES Limited (formerly the Bureau of Sugar Experiment Stations) in Australia. More than 200 hybrid clones of *Saccharum* spp. were preserved at 18°C for 12 months and no phenotypic modifications were observed in the recovered plants. However, with the advancements in tissue culture techniques, *in vitro* preservation became more valuable for the preservation of genetic resources especially of sugarcane [31]. The minimal medium used in *in vitro* preservation has been used successfully during short and medium term preservation, especially for meristems and shoot apices. Decline in explants metabolic activity is usually achieved by changing physical environment or composition of the media used [32]. The commonly used approaches are: lowering of incubation temperature and use of osmotically active compounds such as sorbitol, mannitol and sucrose. Moreover, growth inhibitors like abscisic acid (ABA) is also frequently used. Various factors i.e. vitamins, salts, osmotic stress and others have been explored by different researchers. Survival rate varied in all these experiments but nobody exploited genetic or cytological

studies to assess the genetic stability of *in vitro* plants. For diploid species, Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP) and Inter-Simple Sequence Repeat (ISSR) have been used successfully to assess genetic stability of *in-vitro* plants [33]. But, for plants like sugarcane which has complex polyploid genome, these tools are inappropriate as interpretation of the results become tricky [34]. Using microscopic techniques for sugarcane is also difficult because of small size and large number of chromosomes and also due to the presence of various cytotypes [35]. In this context flow cytometry has got attraction as it ensures estimation of relative amounts of plants nuclear DNA quickly and precisely [36]. Cytometry is able to discriminate between plants derived different culture techniques and has extensively been used, in many economically important species such as *Gossypium hirsutum*, *Vitis vinifera*, *Passiflora* spp., *Elaeis guineensis*, *Musa acuminata* and *Prunus cerasus* [37]. Flow cytometric analysis of shoots was performed after every 6 months of storage. As a consequence, a discrete behavior of tested varieties was observed during storage and on average approximately 80% cultures were able to recover. From these findings it is concluded sugarcane genotypes can be maintained in minimal growth condition for extensive periods but may lead to genetic variations.

## 3. Omics approaches to improve sugarcane crop

The word "Omics" has become a broader term and it is impossible to cover it just in one topic. Omics approaches have explored understandings of complex interactions between genes, proteins and metabolites. These integrated approaches heavily rely on analytical methods, bioinformatics, computational analysis and many other disciplines of biology. Using genomics, proteomics, transcriptomics and metabolomics approaches, the consistency and predictability in plant breeding and transgenic technology has been improved. It has helped to produce high quality and stress resilient crops with enhanced nutritional value in less time and lower input usage. Omics has provided insights into the molecular mechanisms involved in insect resistance and tolerance to herbicides, cold, salinity and drought stresses [38]. To interpret the omics (genomics, transcriptomics, proteomics and metabolomics) approaches in sugarcane for higher yield, higher sucrose contents, biotic and abiotic stress tolerance, one should have knowledge about the genome structure, physiology and functional veracity of sugarcane with other related crops.

### 3.1. Genomics

Sugarcane has a large genome size of 7440 Mb (mega base pairs) having 2n = 100–300. The genome is supposed to be evolved as a result of a complex hybridization event. It is considered that *Saccharum officinarum* is octoploid. The monoploid genome size of *Saccharum officinarum* is 930 Mb and that of *Saccharum spontaneum* is 750 Mb, twice of the size of rice genome (~390 Mb). Geneticists are trying to interpret the associations of complex sugarcane genome with other similar crop plants. The level of genome varies from diploid to decaploid among the *Poaceae* species [39]. The conservation and origin of gene function is suggested by gene order which is maintained by synteny of the genome [40]. The TE (transposable elements) intervening

between coding genes strongly support the extension of genome in grasses. Transposons and retrotransposons are two categories of transposable elements. In plants, the most abundant retroelements are LTR (long terminal repeats) retrotransposons. Transposase proteins are involved in insertion-deletion mechanisms. Active sites of the transcription control the movement of retrotransposons, which reinsert them into the genome after each propagation cycle to increase copy number. Recent studies show that there exists a gene remodeling mechanism which results in the generation of new genes. As a result of gene remodeling, gene expression is altered by new regulatory networks [41]. The study of transposable elements in wheat and barley [42] provided close relationship of transposable elements with genome structure. The transposable elements in sugarcane can be activated and evaluated by functional transcriptomic approaches. The major limitation in sugarcane genetic improvement is its genome size. To sequence the genome of an organism Bacterial Artificial Chromosomes (BAC) are used. BAC (Bacterial Artificial Chromosomes) library was constructed with Hin*d*III partial digestion for sugarcane cultivar 'R570' having more than 100,000 clones with 130 mega base pairs (Mb) insert size [43]. For map-based cloning of sugarcane, BAC resources will be highly esteemed and physical map of sorghum (http://www.genome.clemson.edu/tools/contig_viewer/index.html) will be used as complementary tool.

### 3.2. Transcriptomics

Transcriptomic approaches have emerged as an effective tool for functional characterization of unknown genes. In combination with proteomics and metabolomics, these approaches are very useful for the development of improved sugarcane clones. It reduces the complexity of data and targets. Only active genes in the cell or tissues are considered at the time of sampling. By employing transcriptomic approaches, one can easily compare similar type of tissues at different developmental stages in different organisms growing in different conditions [44].

### 3.3. ESTs

Due to large size and complexity of genome, the whole genome sequence of sugarcane was not available. The genome size of its modern cultivar is considered to be more than 10 GB. From 12th July, 2017, NCBI database has 83,138 GSSs (genome survey sequences), 285,216 ESTs (expressed sequence tags) and 13,382 nucleotide sequences including 491 sequences of mRNA under the search of "*Saccharum*". There are three main groups of ESTs including a large group resulting from a modern variety of sugarcane and two small groups from *Saccharum officinarum* and *Saccharum arundinaceum* (**Table 1**). Majority of ESTs belong to six cultivars from different countries including Australia (Q117), USA (CP72-2086), India (CoS 767, Co 1148) and Brazil (SP80-3280, SP70-1143). Most of the ESTs are from mixed tissue samples of Brazilian varieties i.e. P57150-4 x PB5211 or SP83-5077, RB80-5028; SP80-87432, RB855205, CB47-89, RB845298, SP803280 x SP81-5441, SP80-185, SP80-3280 and SP87-396 [45].

Many projects have been executed for sequencing sugarcane ESTs (expressed sequence tags) in Brazil (http://sucest.lad.ic.unicamp.br/en), South Africa and Australia [46]. Until now more than 0.3 million (300,000) ESTs have been generated. A database holding 0.238 million (238,000) ESTs (constructed from diverse organs and tissues) from 37 libraries was erected by

| *Saccharum* species and hybrids | No. of ESTs | Nucleotide sequences |
|---|---|---|
| *Saccharum officinarum* | 20,701 | 7066 |
| *Saccharum arundinaceum* | 341 | 234 |
| *Saccharum* hybrid cultivar | 284,482 | 2267 |
| Mixed cultivar of *Saccharum* hybrid | 73,778 | 10 |
| SP80-3280 cultivar of *Saccharum* hybrid | 135,534 | 54 |
| CoS 767 cultivar of *Saccharum* hybrid | 25,382 | – |
| Q117 cultivar of *Saccharum* hybrid | 9141 | 54 |
| SP70-1143 cultivar of *Saccharum* hybrid | 24,313 | 8 |
| Co 1148 cultivar of *Saccharum* hybrid | 1069 | 2 |
| CP72-2086 cultivar of *Saccharum* hybrid | 7993 | 4 |
| Co 740 cultivar of *Saccharum* hybrid | 310 | 25 |
| CoC 671 cultivar of *Saccharum* hybrid | 315 | 67 |
| NCo376 cultivar of *Saccharum* hybrid | 535 | 11 |
| H50-7209 cultivar of *Saccharum* hybrid | 27 | 3 |
| F134 cultivar of *Saccharum* hybrid | 4 | – |
| Co 62175 cultivar of *Saccharum* hybrid | 206 | 1 |
| Co 86032 cultivar of *Saccharum* hybrid | 30 | 101 |
| Unknown cultivar of *Saccharum* hybrid | 3904 | 339 |
| **Total** | 285,216 | 13,382 |

**Table 1.** ESTs (Expressed sequence tags) and number of nucleotide sequences corresponding to *Saccharum* species and hybrids submitted in db_EST and db_Nucleotide, respectively (NCBI: 12th July, 2017).

SUCEST (the Brazilian ONSA consortium's sugarcane EST project). More than 43 thousand clusters (that may signify distinctive transcripts) were assembled by cluster analysis of the SUCEST. A BLAST search showed that almost 50% of these expressed sequence tag clones had no resemblance with known proteins. In Genbank, almost 40% of the clones represent full length protein sequences. The genes involved in diverse metabolic processes have successfully been recognized by analysis of SUCEST database. These analyses reveal that assemblage of ESTs is highly illustrative and indicate tagging of thousands of sugarcane genes [47]. ESTs represent gene encoding sequences, natural antisense transcripts, transacting siRNA precursors, miRNA and most commonly noncoding RNA. The information provided by EST dataset is an important starting point to know about the genome of an organism. It can also help to determine genes of agronomic importance (tolerance of biotic and abiotic stresses, sugar content and mineral nutrition). EST availability makes possible the analyses of gene expression on a large scale. Numerous studies have been conducted for *in-silico* analysis of transcript enrichment using different cDNA libraries [48].

### 3.4. Proteomics

Proteomics is the large-scale study of proteome (whole protein contents) and diverse properties of proteins. Through proteomics approaches, we can determine the structural and functional details of biological systems under different conditions. Proteomics has been a major field of functional genomics after the completion of many genome sequencing projects. It has also helped to understand the mode of actions, resistance mechanisms and bio-degradation of pesticides. However, in sugarcane, proteome study is a little bit complicated as no standard protein extraction protocol is available [49]. As compared to other monocots, sugarcane proteomics have not gained momentum yet. Finding protein extraction techniques is a stepping stone in shaping up sugarcane proteomics. Earlier, isoenzyme pattern was used as a tool in sugarcane varietal identification and taxonomy. Isoenzyme pattern was analyzed on 1D gradient polyacrylamide gels based on molecular weight differences [50]. *Saccharum* species (*S. sinense*, *S. edule*, *S. robustum*, *S. spontaneum* and *S. officinarum*) were discriminated from other related genus *Eriochrysis*, *Imperata*, *Narenga*, *Eriochrysis*, *Miscanthus* and *Erianthus* by isozyme pattern of acid phosphatases, leucine aminopeptidases and esterases. O'Farrell [51] introduced 2DE which increased interest in sugarcane proteomics. 2DE technique was then used to study sugarcane roots [52], stalks, leaves [53], meristematic cells and suspension cells [54]. Changes in 2DE protein pattern was observed under different stress conditions. Sample preparation is a crucial step and is necessary for reproducible results. Sugarcane tissues are rigid, fibrous in nature, have sucrose, phenolic compounds and other metabolites in its stalk. Protein extraction protocols have been optimized for the extraction of protein from leaves, meristem and cell suspension cultures but extraction of protein from stalk is still a challenge [55]. Since sugarcane stalk is the core site for sucrose metabolism and host-pathogen interaction but no reproducible protocol is available for the isolation of proteins from stalk tissues.

### 3.5. Metabolomics

Metabolomics is the study of metabolites within the cells, tissues or organism. Proteomics is the study of gene product produced whereas metabolomics explores whether gene products are metabolically active within an organism or not. It also includes role of metabolites in various cellular processes. Hence, metabolites are direct indicators of the performance of a plant under particular biotic or abiotic stresses [56]. Nutritional quality and plant health can be improved by monitoring the changes in metabolite profiling. So, the retrieved informations can effectively be used to develop improved crop varieties as well. Variations in metabolite pattern can also assist to distinguish the mode of action of pesticide which provides critical information for the discovery of new pesticides. Metabolomics may be employed to determine differences and similarities between parents and offsprings on the basis of metabolite composition. Mass spectrometry (MS) and NMR (nuclear magnetic resonance) techniques are used for metabolic profiling, to monitor the metabolic regulations and to analyze the impact of herbicides, pesticides, high temperature, intense light, humidity, soil type, salinity, fertilizers and pests on metabolite composition. One of the fundamental reasons for unavailability of data on sugarcane metabolites is the complexity of sugarcane genome and metabolomes. Most of the research has been focused on differential gene expression. Second constraint is the limited availability of technology due to its sensitivity and labor intensity. Recently,

metabolome (whole metabolites in a specific tissue) has been used as a tool for understanding metabolic regulations. This work was accomplished by some advanced technologies where multiple metabolites were determined in a particular tissue within an hour simultaneously. GCMS (gas chromatography-mass spectrometry) is a vastly used technique that separates the metabolites of different types and identify them on the basis of mass spectral matching and retention time. Identification and extraction methods were optimized for thirty sugarcane metabolites. Hence, metabolome studies are of pivotal importance to understand interaction between the genes and their resultant proteins which can be used to understand mechanisms of sucrose accumulation in sugarcane [57].

## 4. Transgenic approaches for crop improvement

Transgenic technology is the only technology through which alien genes may be introduced across the species. Sugarcane has well be explored to engineer for certain valuable agronomic traits and for enhanced sucrose contents. Most of the transformation events reported in sugarcane are through biolistic, anyhow *Agrobacterium* and electroporation have also been used. Success of engineered lines depend upon the integration and stable expression of introduced gene/s. Recalcitrancy, low transformation efficiency, transgene inactivation and difficult backcrossing are major bottlenecks in sugarcane transformation. Though limited reports are available for the field plantation of transgenic sugarcane yet a large number of research groups are involved in engineering sugarcane genome [58].

### 4.1. Developing genetically modified varieties with improved biotic stress tolerance

#### *4.1.1. Herbicide resistance*

Herbicide resistance is one of the major traits in transgenic plants. It is so desirable that more than 70% of the transgenic crops growing worldwide are herbicide resistant. Various herbicide resistance genes i.e. *EPSPS*, *bar*, *aroA and BXN* have been have been transformed to crop plants for developing herbicide tolerant crops. Crops resistant to glyphosate and glufosinate have been cultivated since 1990s. Plants having ability to tolerate high dose of glyphosate have been developed through biolistic transformation whereas *Agrobacterium* and other methods of transformation (electroporation) have also been tested [59]. Gallo-Meagher and Irvine [60] reported *bar* gene transformation in sugarcane by biolistic gun. Resultant transformants showed tolerance against basta which authenticated effectiveness of *bar* gene not only for herbicide tolerance trait but also as a selectable marker gene, for the selection of putative transformants. Enriquez-Obregon et al. [61] reported transformation of *uid1* and *bar* genes in sugarcane by *Agrobacterium* mediated transformation. They obtained GUS-positive and BASTA resistant calli. Similarly, BASTA resistant variety (Ja 60-5) of sugarcane has also been developed through *Agrobacterium* mediated transformation. Transformation frequencies reached up to 10–35% by employing different transformation protocols. The *PAT* (phosphinothricin acetyltransferase) and *neo* (neomycin phosphotransferase) genes were reported to be transformed in SP80-180 genotype of sugarcane by biolistic

method [62]. The selected transformants were resistant against commercial formulation of ammonium glufosinate. Southern blot analysis was used to confirm the stable integration of *neo* and *PAT* genes. While western blot analysis and RT-qPCR were used to analyze the expression of these genes. Another report was given by Manickavasagam et al. [5]. They developed herbicide tolerant sugarcane plants by *Agrobacterium* mediated transformation. This was first report of *Agrobacterium* mediated transformation in which axillary buds from 6 months old plants were used as explant. *Agrobacterium* with binary vector pGA492 having *β-glucuronidase*, *neomycin phosphotransferase II* and *bar* genes in between the T-DNA regions was used for transformation. This study proved that phosphinothricin (5.0 mg/L) is more effective selective agent as compared with kanamycin and geneticin. Southern blot analysis was used to confirm the transformants. Leibbrandt and Snyman [7] reported the transformation of *pat* gene in NCo 310 genotype of sugarcane which confers resistance to the herbicide Basta. Stable transgene expression was evaluated in glasshouse and field conditions.

#### *4.1.2. Insect resistance*

Insect pests are one of the major yield limiting agents which cause serious losses to crop yield. Economically important insect pests of sugarcane can be categorized into borers, sap sucking pests, white grubs and termites. Sugarcane pests show extensive variation in species composition in different tropic and subtropic agro climatic regions. All around the world, sugarcane is facing problems of insect pests and diseases which are seriously affecting sugar production. No exact estimates are available for these cumulative losses caused by the insect pests and diseases. Anyhow, economic losses caused by certain pests has been estimated. Annual loss of $10–$20 million were estimated to sugar industry at Lower Rio Grande Valley of Texas only by *E. loftini*. Similarly, wooly aphid (*Ceratovacuna lanigera*) has been estimated to cause 18.3% yield losses during sixth months [63]. Most of the sugarcane cultivars growing in the field are outcomes of hybridization and selection. Advancements in molecular biology and genetic transformation have helped researchers to develop transgenic sugarcane plants with desired agronomic traits particularly for insect pest resistance. Different types of molecules have been manipulated to produce insect resistant plants such as lectins, proteinase inhibitors, ribosome inactivating proteins, secondary metabolites, delta endotoxins and insecticidal proteins.

Considerable advancements have been made to develop transgenic sugarcane having resistance against lepidopteran borers such as *E. loftini*, *D. saccharalis*, *S. excerptalis* and *C. infuscatellus* by introducing various cry genes. *Bacillus thuringiensis* derived cry genes encoding toxins have been expressed in sugarcane to engineer resistance against insect pests. First transgenic sugarcane was developed by Arencibia [6] against *D. saccharalis*. Five transformation events were selected exhibiting considerable resistance against borer in spite of very low expression (0.59–1.35 ng/mg of soluble leaf protein) of transgene. Truncated *cry1A(b)* gene was expressed in sugarcane under *CaMV 35S* promoter. Lower level of expression was observed in transgenic plants perhaps because of lower activity of the aforementioned promoter in monocots. Low to medium level internode invasions were also observed in the transgenic lines. Transgenic lines were developed with modified GC contents (37.4–47.5%) of *cry1Ac* gene and effect of change in GC contents, was

observed on the expression of transgene [69]. Transgene expression was determined as 1–10 and 0.2–6.0 ng/mg of total soluble proteins in the leaves and stem respectively, which was seven times higher than reported by Arencibia et al. [64]. Plants also showed better resistance to sugarcane stem borer, when tested by *in vivo* and *in vitro* insect bioassay. Expression of the transgene was further increased with increase in GC content of *cry1Ac* gene and was determined as 2.2–50 ng/mg of total soluble proteins when GC content was increased to 54.8%. Hence expression of *cry1Ac* gene in sugarcane increased with increase in its GC content [65].

Proteinase inhibitors (PIs) derived from both the animals and plants had been introduced in sugarcane to confer resistance against borers. A soybean PI was mixed in the artificial diet of insects and it appeared to have detrimental effects on growth of *D. saccharalis*. Falco and Silva-Filho [66] developed transgenic sugarcane expressing Soybean Kunitz Trypsin Inhibitor (*SKTI*) and Soybean Bowman-Birk Inhibitor (*SBBI*) driven by maize ubi-1 promoter. Transgenic lines were evaluated against *D. saccharalis* by feeding excised leaf tissue and by infecting plants with neonate larvae in the green house. Transgenic lines with *SBBI* did not show significant change in the larvae mortality whereas it was slightly higher when fed on the leaves of *SKTI* expressing plants. Leaves of transgenic plants with *SKTI* or *SBBI* inhibited insect growth and metabolism, hence resulted in reduced insect weight. Dead hearts were also observed in almost all of the plants tested in green house. Without knowing the level of expression, it is very difficult to establish relationship between inhibitor content and dead heart to determine their role in borer resistance. Aportinin appeared to be more effective in inhibting gut proteinases of *S. excerptalis* as compared with those of *C. infuscatellus* and *C. sacchariphagus* indicus. In succeeding studies, aprotinin gene under maize ubi-1 promoter was introduced in sugarcane and transgene expression was determined as 0.16–0.50% of the total soluble leaf proteins. *In vivo* screening assays also revealed very low mortality of *S. excerptalis* larvae but weight of insects was reduced by 99.8% hence insect growth was affected to great extent.

Besides proteinase inhibitors and cry toxins, certain other molecules have also been explored for the effective control of insect pests in sugarcane. Plant derived insecticidal proteins (lectins) are more valuable for the control of insects as compared with bacterial insecticidal proteins. Wheat germ lectin, snowdrop lectin (*Galanthus nivalis* agglutinin, *GNA*) and avidin were used as dietary proteins in bioassays against larvae of sugarcane white grubs. Wheat germ lectins and snowdrop appeared insecticidal and growth inhibitor for *Antitrogus parvulus* larvae. Avidin also appeared to inhibit growth of *A. consanguineus* [67]. Such positive results encouraged researchers to exploit potential of these genes for the control of white grubs. Sétamou et al. [68] included extracts of snowdrop lectin expressing transgenic lines (0.89% in leaf sheath) in the artificial diet (0.47% lectin of total extractable proteins) and examined responses of *D. saccharalis* and *E. loftini* larvae. When artificial diet containing 0.50% of transgenic *GNA* was fed to the larvae of *E. loftini*, a considerable decrease in larvae survival, pupation percentage, adult emergence, pupal weight, longevity and fertility was observed. Transgenic sugarcane expressing lectin under phloem specific RSs-1 (rice sucrose synthase-1) or maize ubi-1 promoters were developed via *Agrobacterium* mediated transformation. Reduced survival rate, development, fertility and feeding was observed in the larvae of *C. lanigera* feeding

on transgenic plants. Aphid population density was decreased up to 60–80%, and even up to 95% in some lines [69]. Maximum resistance was observed when phloem specific promoter (RSs-1) was used in transformation against sucking pests like wooly aphids. Field performance of transgenic lines is dependent on the expression level and stability of transgene. The most critical factors in this regard are: promoter strength, codon usage, gene silencing and site of integration. Rice polyubiquitin (*RUBIQ2*) promoter has proved as the strongest promoter for transgene expression in sugarcane [70]. Anyhow, maize ubi-1 promoter has also extensively been used in sugarcane for the optimal expression of transgenes. Though maize ubi-1 is a constitutive promoter but it does not give same level of expression in all plant parts, e.g. leaves, roots and stem. Keeping in view the tissue specificity and feeding habits of insect pests, it is necessary to use tissue specific or wound inducible promoters which will overexpress insecticidal proteins only in the target tissues and will prove more effective for the control of insect pests.

### 4.2. Developing genetically modified varieties with improved abiotic stress tolerance

Abiotic stresses may alter physiological status of a plant either directly or indirectly by disturbing its metabolism, growth and development. Among abiotic stresses salinity, drought and low temperature are the fundamental factors that significantly influence plant performance. To combat these stresses plant triggers a cascade of physiological and biochemical reactions. Commonly sugars and other osmolytes accumulate in response to abiotic stresses (drought, salinity and low temperature). Water is an essential component for life but its scarcity is increasing day by day throughout the world. Currently almost 65% of fresh water is being used for irrigation, indicating that survival would not be possible without developing drought tolerant varieties in the near future. Sugarcane is a high delta crop and requires heavy irrigation but is now expanding in the regions where water availability is limited, so only drought tolerant varieties can be grown with success [71].

Molecular studies have explored that any of the plant growing in stress conditions strives to withstand those conditions by activating certain stress responsive genes/proteins. They include antioxidant enzymes, late embryogenesis abundant proteins, Arabidopsis *HARDY* genes [72], various transcription factors and certain protease inhibitors. Transcriptomic analyses of sugarcane under drought stress has lead to the identification of stress related genes. These genes should be figured out that how critical they are for stress tolerance. Molecular Systems Biology can be used to characterize regulatory networks using model plant. A novel sugarcane gene *Scdr1* (sugarcane drought responsive 1) was overexpressed in tobacco. Its overexpression resulted in increased tolerance against salinity, drought and oxidative stress as was evident by increased photosynthesis, water content, germination rate, biomass, chlorophyll content and reduced accumulation of ROS. Physiological parameters were also less affected as compared to wild type plants. The relationship between anatomical structure and drought tolerance have also been investigated. In the roots of sugarcane, number of vessels per unit area, more veins, widened vesicles in bulliform cells, thick cuticle and less stomata per unit area have close association with drought tolerance [73]. Drought tolerant varieties have better growth of mechanical tissues around the vascular bundle and their thick-walled cells have higher degree of lignification. Various genes that encode transcription factors *bZIP*, *DREB*, *MYB* [74] and

*RD26* have also been evaluated to enhance stress tolerance in plants. Transcription factors of *MYB* superfamily which are in abundant, also play crucial role in growth and development under stress conditions [75]. Expression profiling of sugarcane was performed under stress conditions to identify abiotic-stress-inducible genes. Wild type Q117 sugarcane plants were exposed to salinity, drought and cold stresses. Variations in the expression level of four genes encoding for galactinol synthase (*GolS*), late embryogenesis abundant protein 3 (*LEA3*), early response to dehydration protein 4 (*ERD4*) and pyrroline-5-carboxylase synthetase (*P5CS*) were evaluated by real-time PCR. *P5CS* and *GolS* were strongly induced under salt stress whereas, *LEA3* and *ERD4* were induced under cold and drought stress respectively. Overexpression of *CBF4* (C-repeat binding factor 4) gene from *Arabidopsis thaliana* in Q117 led to a considerable increase in the expression of *P5CS* and *ERD4* as compared with wild-type plants under normal conditions. Anyhow expression of *LEA3* and *GolS* did not appear to be affected in transgenic plants. These results suggested presence of active abiotic stress-inducible genes in sugarcane and that expression of *Arabidopsis CBF4* gene in sugarcane can activate stress tolerance genes under normal conditions [76].

Plants use complex mechanisms to adapt ionic/osmotic stresses and accumulate compatible solutes to cope with these stress conditions. Salt stress has drastic effects on photosynthetic activity which affects crop production, product quality and of course sucrose accumulation in cane stalk. These stresses disturb homeostasis at cellular level and even at plant level. It is very critical for the plant to maintain low level of toxic ions in the cell but under salt stress, $Na^+$ and $Cl^-$ ions accumulate in the cytoplasm due to their inability to pump them out. The level of $Na^+$ ion is regulated by specific transporters i.e. plasma membrane $Na^+/H^+$ antiporter *SOS1*. When *AVP1* (Arabidopsis Vacuolar Pyrophosphatase) gene was overexpressed in sugarcane by Kumar et al. [77], increased tolerance against drought and salinity was observed in the transgenic plants. Profused and longer roots were observed in transgenic plants as compared to control. Concurrently, survival of transgenic plants under drought and salt stress indicated their increased level of tolerance against drought and salinity. Constitutive expression of *AVP1* gene improved plant growth under different abiotic stresses. Many explanations have been anticipated including better vacuolar ion sequestration, enhanced auxin transport, increased heterotrophic growth, and more sucrose transport from source to sink tissues. Mutant plants which lack functional *AVP1* gene and transgenic plants for *AVP1* were used to evaluate its role. It becomes clear that AVP1 is a protein with multiple functions. Systems Biology can be of great help for the complete understanding of these complex biological networks [78].

### 4.3. Developing genetically modified varieties with improved sugar recovery

Sugarcane has capacity to store more than 25% sucrose of its fresh weight, so a great potential is there to increase sugar recovery. Advancements in biotechnological tools has helped to understand metabolic pathways involved in sucrose accumulation in sugarcane. Enzymes and control points involved in sucrose metabolic pathway, photosynthetic efficiency, degree of phloem loading/unloading, rate of sucrose assimilation and carbon partitioning within the stem and vacuoles are the key targets which needs to be explored for increasing sucrose accumulation. Expression analysis of genes in relation to sucrose accumulation can be of great help to understand role of various genes involved in sucrose metabolism. Until now

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many studies have been reported on the genes involved in sucrose metabolism directly or indirectly. SPS (sucrose phosphate synthase), SPP (sucrose phosphate phosphatase), SuSy (sucrose synthase), HK (hexokinase), FK (fructokinase) VAI (vacuolar acid invertase), NAI (neutral acid invertase), CWI (cell wall invertase), SAI (soluble acid invertase), PFK (ATP dependent phosphofructokinase), PFP (pyrophosphate dependent phosphofructokinase), UDPase (UDP glucose pyrophosphorylase), ADP-G-PP- (ADP-Glucose pyrophosphorylase) and sucrose transporters (SUT1 and SUT4) are the key enzymes involved in sucrose metabolic pathway [79]. Engineering these enzymes through genetic transformation may lead to increased sucrose accumulation and of course sugar recovery (**Figure 2**).

Fructose 6-phosphate 1-phosphotransferase catalyzes the principal reaction of glycolysis i.e. the reversible conversion of pyrophosphate (PPi) and fructose 6-phosphate (Fru 6-P) into inorganic phosphate (Pi) and fructose 1,6-bisphosphate (Fru 1,6-P2). Pyrophosphate

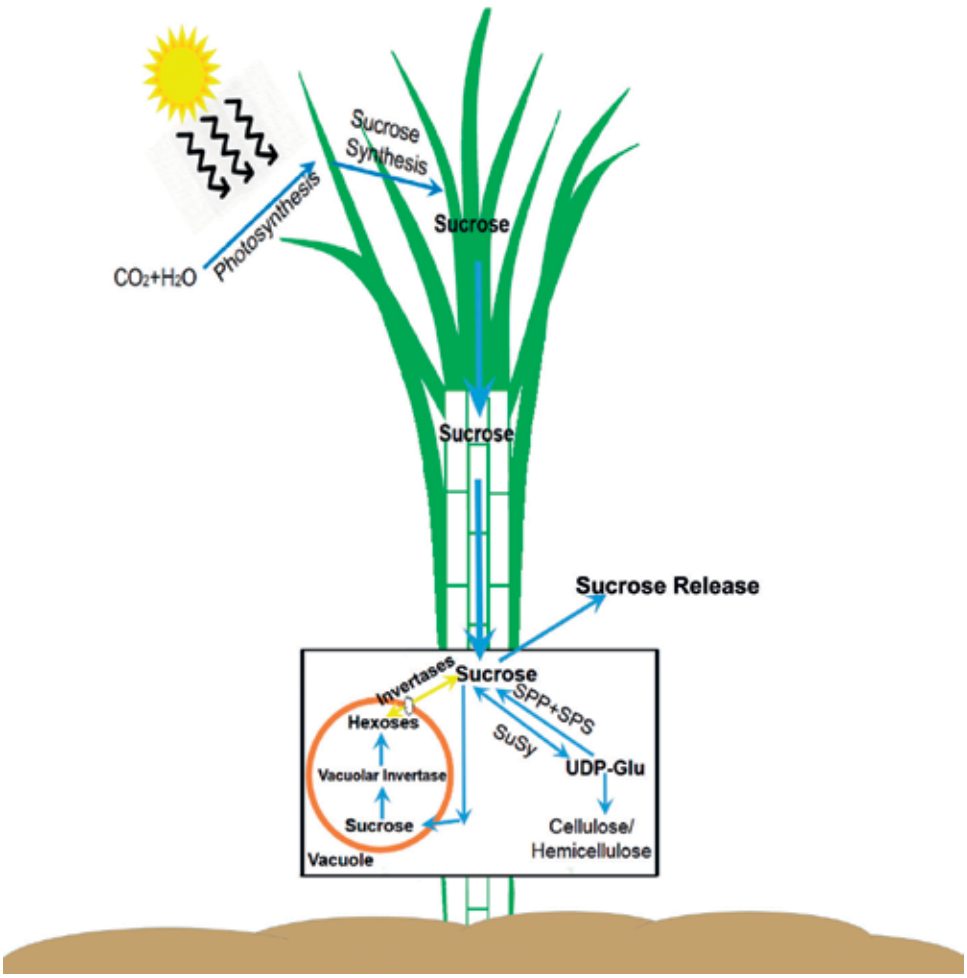


**Figure 2.** Schematic sketch showing the most critical enzymes involved in sucrose accumulation in sugarcane culmn.

dependent phosphofructokinase (PFP) is also partially responsible for being cycled between the hexose phosphate and triose phosphate pools. This cycling was downregulated by constitutive expression of untranslatable and antisense forms of *PFP-b* gene. Approximately 70% activity was decreased in young internodal tissues and no activity was observed in mature tissues. Hendrik and Botha [80] reported decrease in sugar yield as the result of decrease in the PFP activity. A significant increase in sucrose content (in more than 50% of the lines) was observed in the immature internodes, but even 30% downregulation of Pyrophosphate dependent phosphofructokinase (PFP) activity did not affect the mature internodes as compared to wild type. Mature internodes of most of the transgenic lines showed higher sucrose accumulation but was not significant. Hence Pyrophosphate dependent phosphofructokinase (PFP) activity in internodal tissues of sugarcane has a positive relation with respiration and is inversely related with sucrose content. In transgenic plants, no significant difference was observed in development and growth of plants both under greenhouse and field conditions. So PFP (pyrophosphate dependent phosphofructokinase) influences the sucrose accumulation ability of biosynthetically active and young culm of sugarcane. Equilibrium of glycolytic intermediates (stored sucrose) is restored when ATP dependent phosphofructokinase and the PFP activity is sufficient.

Sugarcane culm is an important experimental system to elucidate biochemical and molecular mechanisms involved in sucrose accumulation or carbon partitioning for the application of gene expression studies in this context [81]. Vacuolar targeted expression of sucrose isomerase gene doubled sucrose accumulation in the culm of greenhouse growing plants. Engineered sugarcane plants not only depicted enhanced sucrose transport but also photosynthesis and sink strengths were improved. These results highlighted importance of sugarcane as an energy crop as more carbon source would result in more biofuel production. Higher level of sucrose and accumulation of isomaltulose (a high value sugar) has also been reported in sugarcane [82]. An experimental study was conducted to explore biosynthesis of isomaltulose (IM) through engineering metabolic pathways. Sucrose ($\alpha$-D- glucopyranosyl 1,2-D- fructofuranose) is converted into isomaltulose ($\alpha$-D-glucopyranosyl-1,6-D-fructofuranose) by some bacteria. This sucrose is resistant to several microorganisms as is not metabolized by invertases. Easy digestion (likewise glucose and fructose) by humans is another significant advantage of this sweetener. Instead of salivary invertases, intestinal disaccharidase is involved in the digestion of isomaltulose, so its digestion is relatively slow. Anyhow, it is beneficial because it does not affect insulin and blood glucose levels. Owing to be acariogenic, non-hygroscopic, stable and slowly digestible sweetener, it has mounting market. Biosynthesis of isomaltulose (IM) involves a sucrose isomerase (SI) that does not require cofactor and substrate activation [83]. More isomaltulose (IM) is produced in sugarcane culmn when highly efficient sucrose isomerase (SI) is targeted to vacuole. Further, IM (isomaltulose) could be accumulated without any prominent decrease in sucrose content. Sucrose contents appeared to be doubled in selected transgenic lines but further studies would be required for commercial scale application of this trait i.e. patterns of developmental expression, compartmentation and enzyme stability resulting in high isomaltulose (IM) content. Hence, sucrose isomers can be produced in sugarcane by transgenic technology. Isomaltulose was produced either by expressing sucrose isomerase in the apoplast or in the vacuole. Apoplast-targeted expression did not show any significant

increase in isomaltulose (IM) whereas, vacuole-targeted expression of transgene resulted in significant increase in the isomaltulose (IM) accumulation under greenhouse conditions [91]. Hamerli and Birch [84] reported the first field trial of transgenic sugarcane producing trehalulose (TH). Synthetic sweeteners, an alternative to sucrose are produced through fermentation or chemical reactions which are very expensive. Production of sweeteners in sugarcane through targeted expression of transgene in the mature stem can be an economical alternative. For targeted delivery of proteins into the plastids and vacuoles, transit peptides have already been established in transformants. Zhang et al. [85] worked out not only to develop abiotic stress tolerant sugarcane but also on engineering metabolic pathways for improved trehalose (a valuable sugar moiety) content. Directing sucrose accumulation to vacuole in spite of cytosol may prove an effective strategy for enhanced sucrose accumulation because vacuole occupies large space in the cell. Hence, biotechnological interventions can do a lot to improve sugar recovery in this sweet grass.

## 5. Potential of biotechnology to promote sugarcane as a future energy crop

Plant biomass from grasses including sugarcane, can be used as a renewable source of energy by converting their cellulose, hemicellulose and lignin into bioethanol. Plant derived biofuels reduce dependence on fossil fuels and is of great importance in the countries where oil reserves are limited. Engineering plastid genome of sugarcane may prove a great milestone in this regard [86]. Biofuels produced from plant lignocellulosic biomass (second generation biomass) have advantage over first generation biomass in term of CO2 balance and net energy. Another advantage is that they have no competition for supplies with food industries. As a result, production of bioethanol from 2nd generation biomass is more economical. Sugarcane is one of the most economical source of bioethanol all over the world. Brazil is the leading country in this regard and 50% of the country energy needs are fulfilled by sugarcane ethanol [87]. National fuel alcohol program (ProAlcooL) was launched by Brazil. Major aim of this program was to replace usage of gasoline with bioethanol. In Brazil, 6.19 billion gallons (23.4 billion liters) of ethanol was produced from sugarcane whereas 15% of the total electricity was generated from sugarcane bagasse during 2014 [88]. The genetic foundation of current sugarcane breeding program started with interspecific hybrid varieties originated from early breeding activities in West Indies, India (e.g. Co 206, Co 207) and Indonesia (e.g. POJ 2878, POJ 2364). Sugar yield was increased up to 1–2% per annum by sugarcane programs and most of this increase is attributed to genetics. Conventional breeding in sugarcane has certain limits, as a result desired results could not be achieved. *Saccharum* spp. is genetically complex having 2n = 100–130 with intricate genomic makeup evolved through highly successful interspecific hybridization between *Saccharum spontaneum* and *Saccharum officinarum*, which have well been explored to develop commercial varieties. Ming et al. [89] summarized usage of conventional and molecular approaches for the genetic improvement of sugarcane making it world's most efficient crop in terms of conversion of solar energy into chemical energy.

In sugarcane, about two-third of the photosynthetically fixed carbon is stored in the form of cellulose and hemicellulose. Sugarcane mills produce millions of tons of bagasse annually

in addition to the leaves which are left behind in the field. Sugarcane bagasse is an excellent 2nd generation source for production of ethanol and bioelectricity [90]. High cost of enzymes limit the conversion of hemicellulose and cellulose into cellulosic ethanol. Production cost of enzymes can be decreased by the overexpression of cellulolytic enzymes in GM (genetically modified) plants to meet the demand of sugarcane mills. Adoption of new technologies may help to overcome issues relevant to the stability, storage and overproduction of enzymes in plants. Sainz [91] reported that highly thermostable and hydrolytically efficient enzymes were produced by genetic engineering. Transgenic sugarcane plants overexpressing bacterial endoglucanase (EG) and fungal cellobiohydrolases (CBH I and CBH II) were developed. Targeting EG to chloroplasts and cellobiohydrolases to vacuoles resulted in elevated enzymatic activity in the mature plant leaves. This increased enzyme activity demonstrated that cellulose hydrolytic enzymes can be produced in sugarcane plants [92] and will boost up energy production from cane and its by-products including bagasse. In addition to the traditional agricultural products (food, feed and fiber), plants are emerging as a valuable source of energy, fuel, biomaterials and chemical precursors for the industry. Advancements in research are of pivotal importance to meet the increasing demand of quality raw materials [93]. Genetic engineering techniques are playing important role to achieve this goal and are envisioned to play leading role in the production and processing technology. For instance, input cost can be reduced by producing raw material in plants as plants have proved an effective platform for the production of industrially important compounds. GM microbes are commonly used at industrial scale for rapid conversion of raw materials into desired product. Conversely, a few GM crops have gained commercial status in spite of wide spread eagerness and renowned potential of genetic engineering for crop improvement [94]. Biotechnology occupies a central role in US Department of Energy (DOE) to develop crops with modified cell wall composition. The DOE has received encouraging appreciation because of their research on bioenergy crops and production of valuable processing enzymes by engineering metabolic and biochemical pathways [95]. To successfully attain national goal of bioeconomy, genetic engineering is appearing as a major contributor. A wide range of plants like corn, poplar, switchgrass, canola, sorghum, soybean (*Saccharum* L.) had been used to produce bioenergy but sugarcane is far better choice as is a perennial crop which does not require reseeding after each growing cycle. Hence, sugarcane is the most valuable crop for the production of bioethanol which can further be improved by employing biotechnological innovations.

## 6. Conclusions

Conventional research has contributed to great extent and has delivered its maximum. So, the only hope to get improved agricultural crops is the implication of advanced research. Adoption of biotechnological interventions have proved their worth and more than 18 million farmers in 26 countries are growing GM crops on an area of 185.1 million hectares (457.4 million acres) which is increasing each year. More than 90% of these crops are either insect resistant or herbicide tolerant resulting a massive decrease in the usage of chemical pesticides by 37%, increased crop yields by 22%, and increased farmer's profits by 68%. Though, biotechnological interventions have produced agronomically improved genotypes yet scientists

are currently working to engineer sugarcane crop as a platform for large scale production of chemicals with industrial as well as therapeutic significance. Hence, Biotechnological interventions hold great promises to develop a better sugarcane crop with improved agronomic traits, sugar contents and biofuel production.

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Chapter 8

# Biotechnology of Drought-Tolerant Sugarcane

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Additional information is available at the end of the chapter

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**Abstract**

Water stress exists in most sugarcane cultivation areas, which are not supported by irrigation system and have low rain precipitation. Better understanding of physiological and biochemical mechanism, underlying plants response to water stress, have been achieved to develop drought-tolerant plants by biotechnology approach. To survive and grow normally, plants use a range of strategies to cope the water stress such as changes in gene expression and accumulation of organic compound called compatible solutes. Observation of drought stress response in sugarcane found the presence of a drought-inducible protein called SoDip22 and that the expression was induced by drought stress and ABA hormone treatments. However, the function of this drought-inducible protein has not been elucidated and only suggested that the protein may play an important role in maintenance of water molecule during water deficit state. Biochemical studies on the drought-tolerance mechanism have shown that nontoxic small compound of compatible solute accumulated during water deficit condition. Genetic engineering of glycine betaine (GB), acting as a compatible solute, has been applied for enhancement of water stress tolerance. In sugarcane, bacterial *bet*A gene encodes for choline dehydrogenase (CDH) has successfully introduced and resulted in the transgenic drought-tolerance sugarcane. The CDH converts choline into betaine aldehyde, which is then converted to GB. The overexpression of *bet*A gene increased GB contents that act as an osmoprotectant and help sugarcane acclimate in water deficit condition. This chapter reports the development of biotechnology for drought-tolerant sugarcane.

**Keywords:** sugarcane, transgenic, drought-tolerance, *betA* gene, glycine betaine

## 1. Introduction

Climate changes have been considered as a serious issue in the past few decades and have an impact on the agriculture production and human health. The climate variability and change are projected to result in the frequency of extremely high-temperature events, floods, and

drought conditions. The global increase in temperature is predicted to enhance water losses due to high evapotranspiration rate and resulted in the increase of water stress. Many reports had been published that drought stress can impose decreasing of plants growth and losses in plant productivity. In addition, increasing human population that was predicted by US Census Bureau over 9 million in 2050, will need more demand for food, energy, and the residence. Expansion of city, as a consequence of increasing human occupancy, has significant impact on the displacement of farmland from well-irrigated system toward rain-fed marginal soil which might lose agriculture production.

Sugarcane is a major crop to produce sugar in tropical region and that sugar is extracted from sugarcane stem at sugarcane factory throughout the harvesting session. The stem is shredded, crushed, and pressed to produce juice that is separated from bagasse, the fibrous portion of sugarcane stem. The juice is then clarified and boiled to produce syrup, and through multiple rounds of crystallization to produce sucrose. The remaining black thick syrup called molasses is then separated as a by-product of sugarcane industry. Bagasse has several applications, including generation of power for the mill, papermaking, livestock feed and may be a useful source for production of various derivate of cellulose fiber, and fermentation of bagasse to produce ethanol. Due to still remaining high sugars, the molasses is used for alcohol and other fermentation products as well as a stock feed supplement. The molasses and another by-product called as filter cake are often used as a fertilizer on the cane fields. The various valuable products from sugarcane have put the plant as an economically important plant in tropical region. Increasing sugarcane production and processing would not only increase sugar supply and increase farmers income, but also enhance energy security by using bioethanol from sugarcane production and improve the environment.

Water is one of the most critical environments and plays a crucial role in the life of plants. The availability of water has a potential effect on plant's growth and productivity. The disruption of the plant water status due to drought stress condition reduces the plant survival, growth, and productivity in the environment. In the photosynthetically C4 plant species, sugarcane is considered a plant with high water-uptake efficiency. During day time, the C4 plants can slightly close their stomata to minimize evapotranspiration rate without any effect on photosynthetic carbon assimilation. Although sugarcane needs dry season before harvesting, the plant requires optimum water availability during the vegetative growth. Adequacy water supply during vegetative phase will enhance rapid growth, stem elongation, and internodes formation. On the other hand, limited water availability will stack sugarcane growth and seriously affect on sugar production [1]. Since sugarcane is a valuable crop in the tropical countries that is being used for sugar production and others products such as bioethanol, energy, feed, thus a strategy for development of new sugarcane cultivars tolerance to water stress will be an important issue.

The development of a new plant cultivar could be gained either by conventional breeding program or biotechnology approaches. Although sugarcane improvement by cross breeding program had been successfully implemented, creating a new variety through breeding program is laborious and take times around 12 years or even more. Sugarcane is a complex organism with high ploidy levels and chromosome number of 2n = 80 with a basic chromosome number (x) of 10 [2], and has limitation for the development of new cultivars. The flowering

occurrence under field condition is variable, influenced by variety and environmental conditions such as altitude and day length. Sugarcane is a cross-pollinating species although selfing occurs at low levels [3]. Sugarcane pollen is very small, rapidly desiccated, having a half-life only 12 minutes and no longer viable beyond 35 minutes and is immediately dried. Thus, biotechnological approach is believed to become crucial to overcome the limitations of classical sugarcane breeding. Development of transgenic sugarcane may foster the development for creation of new sugarcane cultivars with various important traits such as drought tolerance, high sucrose content, resistance to diseases, high yield of ethanol and biomass for fuels.

Recently, it has been reviewed that understanding of water stress mechanism in sugarcane from molecular, biochemical, and physiological perspectives will be the most promising strategies for developing the biotechnology [1]. From the physiological perspective, to survive and develop normally, plants adapt to water stress with various strategies including altered gene expression [4] and accumulation of specific compound called compatible solutes such as proline, sugar alcohol, and betaine [5, 6]. Water stress increases the level of ABA, and the hormone involved in the signal transduction of gene expression converting the adaptation to the water stress [7, 8]. The change in water stress-related gene expression associated with sucrose accumulation and the genes encoding enzymes involved in amino acid metabolism have been reported in sugarcane [9, 10]. In addition, Glycine betaine (GB) is a compatible solute that is believed to act as an osmoprotectant and converting plant to adapt to the water stress condition in several plants including sugarcane. Understanding molecular and physiological mechanism on the water stress is a major challenge in developing biotechnology of drought-tolerant sugarcane. The objective of this review is to report the development of biotechnology of drought-tolerant sugarcane using the gene that induces glycine betaine accumulation as well as to summarize an efficient method for genetic transformation method mediated by *Agrobacterium* for sugarcane.

## 2. Physiological and molecular drought stress responses in sugarcane

Water stress is one of the most critical environmental abiotic stresses that affect plant's growth and productivity. It was estimated by the International Water Management Institute that by the year 2025, one third of the world will be occupied with severe water scarcity. Moreover, the climate change will induce competition between the use for human consumption and irrigation, which in turn affects the displacement of agriculture to non-irrigated marginal area that reduced in agricultural productivity. When subjected to water deficit or drought stress, plants undergo alteration in physiological started with reduction in protein synthesis, stomatal conductance and photosynthetic rate. Depending on the plants species, drought stress condition will accumulate the compatible solutes to protect cell from serious damage in drought stress tolerant plants. Under rehydration after mild water deficit, almost every plant can return to normal growth, but if the stress was severe, some plants will not survive and dry.

Sugarcane is photosynthetically classified as C4 plant that adapted well in tropical climate. The C4 plants are often considered to be a better adapted to water limitation environments than most other crops, particularly as they are able to maintain leaf photosynthesis with slightly

stomatal close and increase in water-use efficiency. The C4 photosynthesis is characterized by the presence of phosphoenolpyruvate carboxylase (PEPC) as the primary carboxylation enzyme located in mesophyll cell, and by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) as the secondary carboxylation enzyme located in bundle sheath cells of anatomy C4 leaf. Fixation of $CO_2$ from atmosphere is catalyzed by PEPC in the mesophyll cells to form C4 acid (malate and/or aspartate) which is then transported into the bundle sheath cells. The metabolites transport process generates a much higher concentration of $CO_2$ in the bundle sheath cells than the external atmospheres. This elevated concentration of $CO_2$ at the carboxylation site of Rubisco results in suppression of photorespiration. It was believed that PEPC has high affinity to assimilate $CO_2$ from atmosphere [11, 12] and allow high rates of photosynthetic carbon assimilation to occur when stomata are slightly closed to prevent evapotranspiration. This PEPC has a significant role in C4 photosynthesis carbon assimilation and is regulated by environmental conditions such as light [13], water stress [14], and nitrogen availability [15]. Although limited reports, the enzymes involved in $CO_2$ concentration mechanism in C4 plant are considered to play significant role in water resistant [16, 17].

Sugarcane is an important crop to produce sugar and dry condition is commonly observed in sugarcane farm in tropical agriculture. Dry season or drying prior to harvest in irrigated sugarcane cultivation is an important strategy to enhance sucrose content in stem [18, 19]. Gradual water deficit during sugarcane maturation reduce stem elongation and leaf development, but more sucrose become available for storage in stem [18]. In the pathway of sucrose biosynthesis, sucrose-phosphate synthase (SPS) is believed to be a key enzyme for sucrose synthesis in plants [20]. A comparison study on the sucrose content in sugarcane cultivated in different agro-climate showed that dry-land cultivated sugarcane accumulated more sugar compared with wet-land and observation in *Saccharum* species showed that sucrose contents are fluctuated according to the SPS activities [21]. Further physiological study on drought stress revealed that stop watering increased sucrose-phosphate synthase (SPS) along with sucrose accumulation in sugarcane leaf (**Figure 1A**). Similar results were reported that water stress resulted in a stimulation of sucrose synthesis by activation of sucrose-phosphate synthase in spinach [22] and wheat [23]. Addition of ABA increased the SPS activities but did not increase other proteins levels (**Figure 1B**) since the hormone is involved in the signal transduction of gene expression conferring the adaptation [7, 8]. Identification of amino acid residue serine that is responsible for water-stress regulation by phosphorylation mechanism clearly showed that the amino acid is conserved in sugarcane SPS [24]. This experimental result suggests that drought stress induce sucrose accumulation in sugarcane as a mechanism helping the plant adapted to drought conditions. Moreover, overexpression of the gene for SPS has been reported to enhance SPS activity as well as sucrose accumulation in transgenic tomato [25]. The increasing of sucrose accumulation due to overexpression of the gene for SPS enhanced drought stress-tolerance will be an important study to be conducted in sugarcane.

Drought stress induces a wide range of physiological and biochemical responses in plants, including alteration in gene expression. The change in gene expression was triggered both by ABA-dependent and ABA-independent regulatory mechanism. Furthermore, identification by microarray analysis had classified two groups of drought-inducible genes in *Arabidopsis*. The first group is genes encoding for proteins with the function in abiotic stress tolerance and the second group is comprised of regulatory protein such as various transcription factors

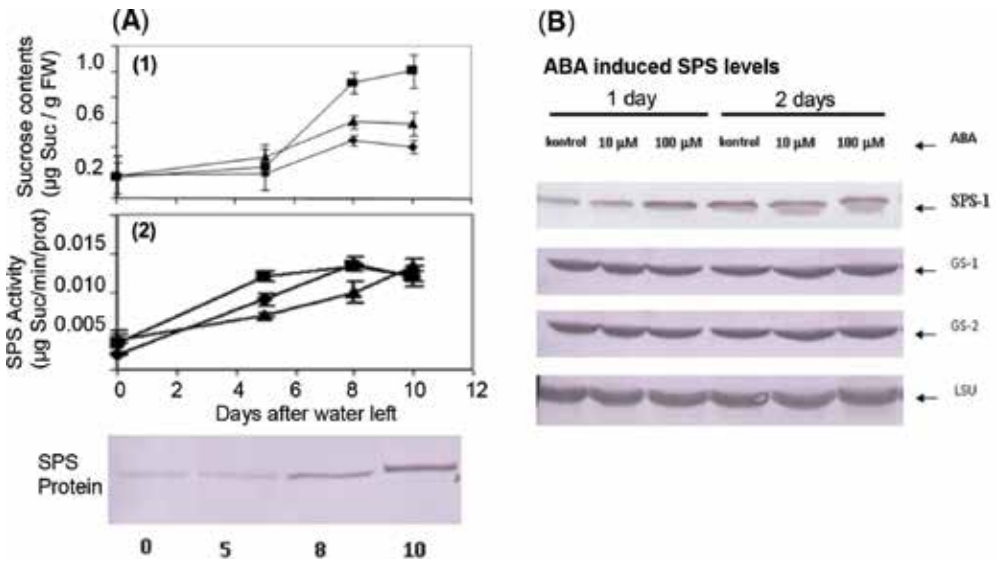


**Figure 1.** Enhancement of SPS levels in sugarcane leaves after drought stress (A) and ABA hormone (B) treatments. Two-months old sugarcane plants grown in green house were treated by either drought stress or ABA hormone. The drought stress was initiated by left sugarcane plants without watering and the SPS activity, SPS protein levels, and sucrose contents were measured at indicated times (A). (B) The fully developed youngest leafs were sprayed with ABA solution at indicated concentration for 1 and 2 days. Total proteins were extracted from the fully developed youngest leaves and the SPS activity was measured according to the method described in [21]. The levels of SPS, Rubisco-LSU, and GS (glutamine synthetase) proteins were detected by Western Blot analysis with specific polyclonal antibody against the proteins. The sucrose was extracted from the leaves using mixture of methanol-chloroform-water, and the sucrose content measured using HLPC. The figures were provided by Dr. Yudhi Rinanto.

and regulation of signal transduction [4]. Molecular study on the responses of sugarcane to drought stress found the presence of a drought inducible protein named SoDIP22 in the water stress tolerant phenotype of sugarcane [10]. A computer search of protein databases revealed that the sequence of the drought inducible protein exhibited significant similarity to that of members of the ABA stress and ripening-inducible (Asr) protein family, such as 73% identical to rice OsAsr1 protein. The expression of the drought-inducible from sugarcane SoDip22 protein was induced by drought stress and osmotic stress at −0.9 Mpa generated with PEG 6000 and 0.6 M mannitol. The expression of SoDip22 was controlled by the signal transduction pathway through ABA, since exogenous addition of ABA induced the SoDip22 expression, but not other growth regulators. Although the molecular size of SoDip22 was a small protein, only 22 kDa, and has similarity with Asr protein found in the nucleosome fraction which is predicted as a transcription factor, observation of transient expression of the SoDip22 protein did not support the nuclear localization. Interestingly, the protein was inclusively detected in bundle sheath cell of sugarcane leafs and the protein function is predicted to play an important role in the maintenance of water molecule during water deficit in the bundle sheath cell.

Water deficit causes various changes in biochemical reactions, including the production of a complex variety of secondary metabolites. Water stress induces the accumulation of reactive oxygen species (ROS) in plants which are highly reactive or toxic that causes damage to

cellular component such as proteins, lipids, carbohydrate, and DNA. The ROS also controls many processes such as cell cycle and programmed cell death [26]. Exposure of plants to drought condition increases production of ROS such as free radical ($O_2$*, superoxide radicals, OH* hydroxyl radical, $HO_2$* perhydroxy radical) and non-radical forms ($H_2O_2$, hydrogen peroxide and $O_2$, singlet oxygen). To ensure survival under drought stress condition, plants have developed efficient antioxidant machinery that is able to scavenger and detoxify ROS [27]. Plants possess enzymatic and non-enzymatic antioxidant defense system to protect plant cell from oxidative stress by scavenging ROS. The enzymatic activity such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and non-enzymatic antioxidants such as ascorbic acid, reduced glutathione, phenolic, alkaloids, and amino acids can work together to scavenge ROS. Water deficit induced the change activities of SOD, CAT, APX, and GR, according to variety and stress intensity in sugarcane. Drought-tolerant sugarcane exhibited higher CAT and APX activities in the early stage of drought, while the activity of GR was highest at the end of drought stress period compared with drought-sensitive sugarcane [28]. The increase of SOD, CAT and APX under drought stress was further confirmed in most tested sugarcane cultivars [29]. Thus, the activities of ROS scavenging enzymes may be used as a marker of water stress tolerant sugarcane.

Many plants respond to water deficit stress by accumulating non-enzymatic antioxidants defense system to protect from oxidative damage by ROS. Ascorbic acid is one of antioxidants that prevent or minimize the damage caused by ROS. The ascorbic acid has ability to donate electrons in numerous reactions and protect the cell membranes by scavenging the superoxide radical and hydroxyl radical [27]. Furthermore, glutathione is another important antioxidant that is capable of preventing damage caused by ROS. Glutathione exists either in reduced or in oxidized form and it is a crucial metabolite to perform multiple functions including plant responses modulation under abiotic and biotic stresses [30]. Despite ROS scavenging enzymatic and non-enzymatic antioxidants which had been reported to enhance drought tolerance in several transgenic plants [27], their application for developing drought-tolerant sugarcane is still meager.

The accumulation of non-toxic small molecule metabolites referred as compatible solutes or osmoprotectant such as sugar, proline and betaines help plants to survive under osmotic stress [5, 31]. These metabolites may have a role to protect cell membrane and maintain osmotic potential. Studies at physiological, biochemical, and molecular levels suggest that compatibles solutes perform important function in adjustment of plant against salinity and drought stress. Sugar and sugar alcohols have been accepted as osmoprotectant that provide membrane protection and scavenging ROS. The higher accumulation of sugar, such as trehalose, fructans, sucrose, acts as osmoprotectant under drought stress in plants [32]. Sugarcane can accumulate high content of sucrose in storage parenchyma of stem cell that may create osmotic gradient and act as osmoprotectant. Under water deficit, there was a change in stress-related gene expression and sucrose accumulation, but the mechanism responding to the water stress was different. Some genes expression such as genes encoding for asparagine synthase (AS), biosynthesis of proline (OAT), sugar transports were positively correlated, but the genes encoding for proline biosynthetic pathway (P5CS) and the bZIP transcription factor TF1 were negatively correlated with sucrose contents in sugarcane mature culm. The proline content was increased under water deficit condition, but was negatively correlated with sucrose concentration and suggested that proline has no osmoprotectant role in sugarcane [9].

Although the role of proline on osmotolerance remains controversial in plants, evaluation of transgenic sugarcane overexpressing heterologous *P5CS* gene indicated that proline content significantly increases after 9 days without watering. However, the increasing proline content has no effect on the osmotic adjustment, and the proline may protect sugarcane against the oxidative stress caused by water deficit. The results suggested that proline accumulation acts as a component of antioxidative defense system rather than as an osmotic adjustment [33].

The glycine betaine (GB) is one of the compatible solutes and an amphoteric quaternary amine that is considered as the most compatible solute that plays an important role in protecting plants under environmental stress [5, 34]. The GB is synthesized by plants at various capacity, such as spinach and barley accumulate high of GB, whereas *Arabidopsis* and tobacco do not synthesize GB. The role of GB is not only allowing cell to adjust the osmotic potential in their cytoplasm to maintain an appropriate water content, but also protecting protein from the water stress dissociation [34]. When plant cell is exposed to water stress or salt stress, GB stabilizes the structure of macromolecule, helping in maintaining the integrity and proper function of the membrane. Although the detail of the role of GB has not been firmly established, the objective of this book chapter is directed for developing of biotechnology of drought-tolerant sugarcane using production of GB in transgenic sugarcane.

Genetic transformation has a potential role to introduce a new trait in plant cell, including the introduction of new pathway for the biosynthesis of compatible solutes and resulting in transgenic plant with improved tolerance to environmental stress. There are many techniques for introducing a new gene into plant cell such as direct transformation using particle bombardments, micro injection or electroporation and indirect transformation using *Agrobacterium* as the vector. Depending on the plant species, *Agrobacterium*-mediated transformation is well established in dicotyledonous plants and less in monocotyledonous plants. The development of *Agrobacterium*-mediated transformation was successfully established for routine genetic transformation in monocotyledonous plants such as rice, maize, and sugarcane.

## 3. Development of an efficient method for *Agrobacterium*-mediated transformation for sugarcane

The genetic improvement of sugarcane cultivars has been achieved to increase sugar productivity with the cultivars created by conventional breeding. The sugarcane (*Saccharum officinarum*) cultivars contributed high sucrose production and the backcrosses of *S. officinarum* resulted in elite cultivars of *S. hybrid* with higher yield, improving ratooning ability and disease resistance. However, these improvise achievements would still enable the sugar industries to face current issues on climate changes as well as higher sugar demand due to human population growth. Sugarcane has a highly complex genome, low fertilization and tiny seed which make traditional breeding highly difficult and laborious to create new sugarcane cultivars. Recent progress on molecular techniques could be used in sugarcane varietal improvement as well as the combination between both traditional and molecular breeding. Beginning with development of cell and tissue culture of sugarcane that has emerged as a valuable tool for various research activity in sugarcane improvement and propagation,

sugarcane biotechnology has been used to introduce new traits that would be very difficult to conduct with conventional methods or almost not possible. The first establishment of genetic transformation method in sugarcane by Bower and Birch [35] and followed by engineering of agronomic traits using the method into various sugarcane genotypes are the important steps to support the development of sugarcane biotechnology. In addition, consideration also has been given to the development of molecular marker technologies for sugarcane breeding and variety identification [36].

Plant cell has a totipotency ability to regenerate and differentiate into whole plant that completed with leafs, stem and root. The totipotency ability has been used for plant multiplication or micropropagation by inducing meristematic plant tissue in the medium supplemented with plant growth regulator to produce somatic embryogenesis callus, which in turn regenerate into whole plants. In sugarcane, the somatic embryogenesis callus is produced by induction of meristematic leaf tissue on the MS (Murashige and Skoog) medium containing 2,4-dichlorophenoxyacetic acid (2,4 D), and the embryogenic callus is then regenerated into whole plant on the MS free hormone [37]. Thus, considerable effort has been expended to use this micropropagation technique for providing the demand of numerous sugarcane seed. However, the application of tissue culture through somatic embryogenesis induces somaclonal variation in sugarcane [38, 39] that causes variants of phenotype, although it will be reverted to original parental thereafter [38]. Moreover, the presence of somaclonal variation is frequently used to obtain new type of sugarcane cultivars such as resistance to Fiji disease and mildew [40] and resistance to eyespot disease [41]. Although there is wide diversity of the usage, the somatic embryogenesis has been widely used as a part in genetic transformation system for the improvement of sugarcane cultivars [35].

Micropropagation of sugarcane can be also performed by direct regeneration of both apical and axillary meristem buds. The regeneration from axillary buds reduces the somaclonal variation events and is routinely used for mass multiplication of sugarcane [42, 43]. However, axillary buds isolated from field grown sugarcane stalk were frequently contaminated with bacteria and should use unexpected strong sterilant such as mercury chloride ($HgCl_2$) before cultured on MS media. Thus, the proper concentration should be carefully selected since this $HgCl_2$ sterilant is extremely harmful. Alternatively, *in vitro* sugarcane shoot can be regenerated from meristematic shoot apical and multiplied on MS media. There are various methods to improve the multiplication sugarcane shoot *in vitro* using MS media. Using temporary immersion system, shoot formation for sugarcane micropropagation was improved [44]. The shoot induction and multiplication on MS containing higher benzylamino purine (BAP) hormone resulted in weak, tiny, and nonseparable shoots, whereas the lower concentration resulted in desirable quality, well grown, easily separable, and healthy plantlets [45]. The media composition is considered to play an important role for achieving maximum growth rates on sugarcane tissue culture. Amino acids mixtures such as glutamine, glycine, asparagine, cysteine, and casein hydrolysate are frequently used as source of organic nitrogen in culture media [46]. Recently, addition of the amino acids mixture to the MS media has been examined in shoot multiplication of sugarcane. Addition of 2 ppm of glycine did not induce shoot multiplication, and 100 ppm of glutamine gave induction of shoot regeneration, but shoot growth rate was low. However, addition of the combination of glycine and glutamine resulted in well growth and healthy sugarcane plantlets. These results indicate that inclusion

of amino acids of glutamine and glycine in the media for stimulating multiplication of healthy sugarcane shoot will be suitable for future use in micropropagation as well as genetic transformation method.

Genetic transformation technology serves as a useful and practical tool to introduce particular traits for crop improvement. Several genetic transformation methods have been attempted for delivery and expression of transgenes in plants. First genetic transformation methods for sugarcane were developed by direct introduction of desired genes using electroporation [47], polyethylene glycol (PEG) treatment [48], and particle bombardment [49]. These methods were considered less efficient compared to the indirect genetic transformation using *Agrobacterium*-mediated transformation. Furthermore, the direct transformation systems have certain limitations such as requires specific equipment, needs skill labor and results in high copy number integration in the plant genome [50]. The multiple gene copies frequently cause multiple gene arrangement, co-suppression and gene silencing [51]. Although, *Agrobacterium*-mediated transformation is restricted applicable in dicotyledonous plants due to the strictness of the host range of this bacteria, recent research indicated that *Agrobacterium*-mediated transformation is also possible in monocotyledonous plants such as in rice [52], maize [53] and banana [54]. In maize, the *Agrobacterium*-mediated transformation has been used for routine transformation using standard binary vector system and average stable transformation efficiency was 5.5% [55]. The evidences of the *Agrobacterium*-mediated transformation system were also reported in sugarcane using meristematic explants [56, 57]. The *Agrobacterium*-mediated transformation method offers several advantages such as technical simplicity, low copy number and minimal genome rearrangement. Although *Agrobacterium*-mediated method has been applied also to sugarcane, the lack of reproducible results has been an obstacle to establish effective transformation protocol for routine genetic manipulation in the plants. The cell is being traumatic due to *Agrobacterium* infection and poor survival rate thereafter. Oxidative burst, phenolization, and subsequent cell death are frequent phenomena after the *Agrobacterium* infection [58]. Development of the *Agrobacterium*-mediated transformation is necessary to have reproducible and efficient methods in sugarcane. This section demonstrates an efficient transformation system for sugarcane using explant *in vitro* shoot generated from apical shoot tips to minimize bacterial contamination as well as somaclonal variation.

Genetic transformation system has been developed for sugarcane with distinct agronomically important traits, transformation methods, explant and culture condition. However, the use of *Agrobacterium*-mediated transformation method that considered more efficient was limited applying in sugarcane [58, 59]. Moreover, embryogenic callus was mostly reported as the explant for the *Agrobacterium*-mediated transformation system, but the use of callus includes the somaclonal variation. Direct regeneration from explants without an intervening callus phase has several advantages for *Agrobacterium*-mediated transformation in sugarcane. The isolated axillary bud explants from 6-months old field grown sugarcane were infected with *Agrobacterium* harboring the T-DNA of binary vector and resulted in stable transgenic sugarcane. The results suggested that the method can be achieved to generate transgenic sugarcane in about 5 months with transformation efficiency as high as 50% [42]. However, this transformation system needs numerous axillary buds as explants that should be isolated from sugarcane stalk and it is very difficult to avoid bacterial contaminant in the tissue culture

media. By regeneration of *in vitro* shoot using meristematic shoot apical, subsequent multiplication in appropriate MS media will be suitable to overcome the problem of contaminant. The protocol for regeneration of *in vitro* shoot from shoot apical has been developed and with the method, the healthy shoot was rapidly multiplied in the MS media containing additional amino acid mixture of glutamine and glycine (**Figure 2A–F**). Green and healthy *in vitro* shoot from 2 to 4 weeks cultured was separated and basal segment that contains meristematic tissue was excised around 0.2–0.3 cm from the base (**Figure 2B** upper). These basal segments were injured with needles and used as the explant for *Agrobacterium*-mediated transformation. The injured or wounded tissue was suitable to induce *Agrobacterium* infection and allowed the *Agrobacterium* to penetrate into inner meristematic tissue of the basal segment. The presence of meristematic tissues provides young regenerable material that actively divided cell, competent for *Agrobacterium* infection, and improves the adhesion of *Agrobacterium* during co-cultivation [57]. Transient expression analysis showed that *Gus* gene expression was predominantly observed in the basal portion which was injured and contains meristematic tissue (**Figure 2B** lower). After cultured on selection medium containing the appropriate antibiotic for 2–3 weeks, the basal segment regenerates new axillar shoots, in which some of them become albino and bleached due to the presence of antibiotic in the media or regenerated green shoots. The explants with green shoots were transferred to the fresh selection medium and, after 5 times successive cycle, the putative transformant were acclimated. With this method, co-cultivation and antibiotic selection of putative transgenic shoot can be achieved in less than 4 months with transformation efficiencies around 6% when using 2 weeks-old shoot explant and the efficiencies sharply increased as high as 40% when using 4 weeks-old shoot explant. Genomic PCR and Southern Blot analysis indicated that most of the putative transformants contain insertion of the targeted DNA. All together these results suggest that basal segment of *in vitro* sugarcane shoot provides an effective explant for routinely *Agrobacterium*-mediated transformation protocol and produces transgenic sugarcane.

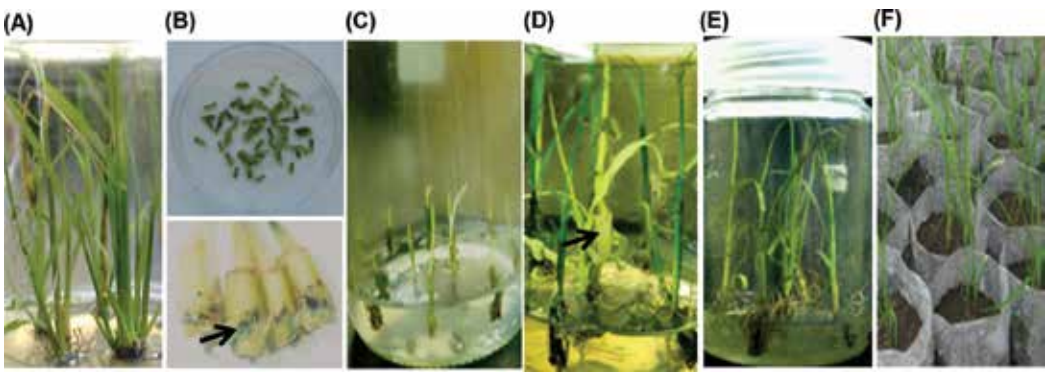


**Figure 2.** Workflow of the *Agrobacterium*-mediated transformation using explant base segments of *in vitro* sugarcane. Green and healthy sugarcane *in vitro* shoot were micro-propagated in MS media and used as source of explants (A). Excised base segments of *in vitro* sugarcane were used as explant for the transformation (B upper) and clear blue spots of *Gus* gene expression were observed in the basal segments (B lower). Elimination of non-transformant and multiplication of putative transformant shoot in the selection MS media containing appropriate antibiotic (C and D). After five cycles in the selection media (E), the putative transformant were acclimated in greenhouse for further analysis (F). Arrow represents clear blue spots in the basal segment and albino shoot of non-transformant.

The achievement of the current sugarcane transformation technology still needs further development. A number of undetermined conditions such as DNA promoter that drives the gene expression, selectable marker, *Agrobacterium* strain and some other factors are becoming important for improving genetic transformation efficiency. The promoter is a key DNA regulatory element that directs appropriate strength and pattern of gene expression in a constitutive or specific manner. Therefore, the promoter plays a crucial role in determining the transformation efficiency. There are some types of DNA promoters that drive strong, constitutive, or organ specificity expression. For example, the viral Cauliflower Mosaic Virus 35S (CaMV 35S) promoter has been widely used in the transformation of many dicot and monocot plants. However, it has been demonstrated that the expression activity of the 35S promoter was low in sugarcane [60]. The rice actin1 and Emu elements have shown to drive higher expression activity than CaMV 35S in different sugarcane tissues [61] and from the current research, it appeared that ubiquitin promoter has an emerging promoter for constitutive expression in sugarcane. The experiment concerning an effective promoter for sugarcane transformation has been also conducted using rice ubiquitin promoter (RUBQ2). The use of RUBQ2 promoter has increased transgene expression by about 1.6-fold over maize ubiquitin promoter in sugarcane [62]. Comparison study on GUS expression driven by CaMV35S and RUBQ2 promoter showed that RUBQ2 promoter produced high level GUS activity with clear blue spot in embryogenic callus and suspension cultures, while the CaMV35S promoter was not detected. Controversially, the GUS expression driven by sugarcane polyubiquitin promoter was dropped to very low or undetectable levels in the transgenic plants resulted from post-transcriptional gene silencing.

Among the factors considered as limiting the recovery of transgenic plant is the involvement of selection marker in genetic transformation system. The selection of genetically transformed cell can be conducted through positive selection and negative selection. The positive selection is referred as those that promote the growth of transformed tissue and negative selection is the use selective agents, killing or fully inhibiting the growth of untransformed cell [63]. The use of gene for selectable marker in combination with targeted gene is directed to identify and allow surviving the transformed cell, and inhibit the growth of non-transformed cell in the media containing appropriate selective agents. Therefore, the use of selectable marker provides easy protocol to support proliferation of transformed cell and remove the un-transformed cell. Among the widely used selectable markers, the genes responsible for resistance of antibiotic kanamycin (*nptII*), hygromycin (*hptII*) and herbicide resistance (*bar*) are frequently applied to develop transgenic plants. The genes of *nptII*, *hptII* and *bar* inactivate the enzymes that play in role of antibiotic resistances neomycin phosphotransferase, hygromycin phosphotransferse and phosphinothricin acetyltransferase, respectively. Determination of the explant sensitivity to the antibiotic and the antibiotic concentration can be potentially effects of the successful genetic transformation. Exceeding high level of antibiotic is not only to kill the nontransformed cell, but also to give retardation for the growth of the transformed cell [64]. Evaluation of resistance to antibiotic kanamycin and hygromycin showed that the antibiotics can be used as selectable marker to obtain stable transformants in the cell suspension culture of the Gramineae such as *Triticum monococcum*, *Panicum maximum*, and *Saccharum officinarum* [65]. Comparison study on grapevine transformation revealed that both antibiotic kanamycin and hygromycin inhibited growth of the untransformed explant at 16 and 1 ug/mL, respectively. Due to the hygromycin which can be applied at lower level than kanamycin, hygromycin appears an appropriate

selective agent [66]. Similar results reported that hygromycin is an effective selective marker for genetic transformation for monocot plants such as rice [67, 68], maize [69], and banana [70]. The herbicide Basta (*bar* gene) has been used as the selection marker for genetic transformation in rice [71], fescue—*Festuca arundinacea* [72], and oil palm [73]. However, limited reports have published the use of both *nptII*, *hptII*, and *bar* genes as effective selectable for sugarcane transformation. Using direct transformation with microprojectile bombardment, stable transformant was obtained after the selection of explant sugarcane callus on the media containing kanamycin and stepped increases in the antibiotic concentration allowed an active growing of callus, plantlets and completely inhibited untransformed callus [35]. When *Agrobacterium*-mediated transformation was performed, similar results were observed using explant callus and *nptII* gene as the selectable marker. Regeneration transformant was successfully conducted by culturing the explant callus on the media containing 150 mg/L paromomycin sulfate [74]. However, the first successful report on *Agrobacterium*-mediated transformation for sugarcane used selectable marker *hptII* gene [57]. There are many reasons to elaborate the discrepancy between the effectiveness of selectable markers *nptII* or *hptII* genes, but hygromycin is much more effective than kanamycin for the selection of transformed cell and at low concentration, the hygromycin provides strong discrimination between transformed and nontransformed cell. Thus, hygromycin at the concentration of 25 mg/L is sufficient for routinely used *Agrobacterium*-mediated transformation for sugarcane.

*Agrobacterium* strain and density frequently have an impact on the plant genetic transformation efficiency. There are many *Agrobacterium* strains used for genetic transformation in plant and, among of them, the LBA4404 *Agrobacterium* strain is widely used for genetic transformation. The LBA4404 has a higher transformation efficiency in several plants such as in tobacco [75], wheat [76], and herb of *Bacopa monnieri* [77]. However, GV3101 *Agrobacterium* strain has been reported with highest transformation rate than EHA105, AGL1, and MP90 *Agrobacterium* strains in tomato [78]. In addition, concentration of *Agrobacterium* and wounding explants are also considered as the factors influencing transgene expression in loblolly pine [79]. Concentration of *Agrobacterium* at $OD_{600} = 0.5$ improved the efficiency of transformation in cotton [80], whereas higher concentration will result in *Agrobacterium* overgrowth and difficulty to eliminate after co-cultivation. In sugarcane transformation system using explant *in vitro* shoot, GV3101 *Agrobacterium* concentration at 0.5 $OD_{600}$ and wounding explants can be used for routinely transformation.

## 4. Genetic engineering of glycine betaine (GB) synthesis improves drought tolerance in sugarcane

Glycine betaine (*N*,*N*,*N*-trimethyl glycine) is an amphoteric small organic compound, highly soluble and do not interfere with cellular metabolism even at high concentration. The molecular character of GB can interact with macromolecule such as enzyme, protein complexes, and cell membrane when cell is exposed to stress condition. Glycine betaine stabilizes the structure and activity of enzymes and proteins, and maintains integrity of membrane against damage caused by environmental stresses [81]. This GB accumulates in a variety of plant species in response to water stresses for osmotic adjustment. Depending on the plant species,

some plant species are accumulator of glycine betaine such as *Amaranthus*, sorghum, sugar beet and the other non-accumulators such as rice, sweet potato, and tobacco [5, 34]. Drought stress enhances accumulation of GB in the accumulator species for osmotic adjustment [82, 83]. Glycine betaine protects the plant cell by acting as an osmolyte, stabilizes protein and membrane cell, and maintains water balance during drought stress. It is widely accepted that the accumulation of GB plays an important role for the acclimatization of plant cell to drought stress. In many plants that do not accumulate GB, application of GB may help reduce adverse effects of the environment stress. The exogenous application of GB at 10 mM improved growth, leaf water content, and net photosynthesis, and increased growth and crop yield under environmental stress [84, 85]. However, consideration of economic and streamline useful application needs to be investigated. Determination of the GB concentration, timing, frequency of application, and a possibility of other disadvantage of exogenous GB application such as the risk of increasing pathogen attack should be well established [85]. Thus, genetic engineering for economically important crops such as rice, maize, and sugarcane that naturally are unable to accumulate GB will be an important target to improve.

Glycine betaine is an osmoprotectant found in wide range of microorganisms, plants, and animals that are synthesized under various environmental stresses [5]. Glycine betaine is mainly synthesized from choline as the substrate through two-step reactions, dehydrogenation of choline, and oxygenation of betaine aldehyde (**Figure 3**). In higher plants, choline is converted by choline monoxygenase (CMO) to betaine aldehyde, and then converted into glycine betaine by betaine aldehyde dehydrogenase (BADH) to GB [34, 86]. In microorganism and mammalian cells, GB is also synthesized by two-step pathway, but choline is converted to betaine aldehyde by choline dehydrogenase (CDH) and not by CMO, and then to GB by same BADH activity [34, 87]. In contrast, a single step-reaction catalyzed by choline oxidase (COD) for synthesis of GB is found in some microorganism such as *Arthrobacter globiformis* and *Arthrobacter panescens* [88] . In addition, a distinct substrate for GB synthesis is found in two halophytic microorganism *Actinopolyspora halophila* and *Ectothiorhodospira halochloris*. The GB is synthesized from substrate glycine by glycine methylation pathway [89]. The increasing knowledge of physiological pathway for GB biosynthesis as well as genomic engineering technology allow to create transgenic plants that are properly tolerant to drought stress by engineering of glycine betaine biosynthesis.

The gene involved in the biochemical pathway can be used either to increase or diminish metabolite product by overexpressing or silencing the gene responsible for the metabolism. In the case of metabolite engineering of GB, the enzymes involved in the biochemical pathway have been focused as a potential target to engineer the content in the non-accumulator plants. For that reason, the genes encoding for the enzymes involved in pathway of GB biosynthesis have been cloned from various microorganisms and plants that accumulate GB. In microorganism, gene encoding CDH (*bet*A) and BADH (*bet*B) have been isolated from *Escherichia coli* [90, 91] and from salt-tolerant bacteria *Halomonas elongata* [92], whereas gene encoding COD or COX was cloned from soil-living bacteria *Arthrobacter panescens* and *Arthrobacter globiformis* [93, 94]. In higher plants, limited number of genes encoding CMO in combination with BADH has been cloned from spinach [95], sugar beet, and amaranth [96]. Genes responsible for GB synthesis from microorganism have become a major target in the genetic engineering of water stress-tolerant in plants that are unable to accumulate GB, such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), rice (*Oryza sativa*),

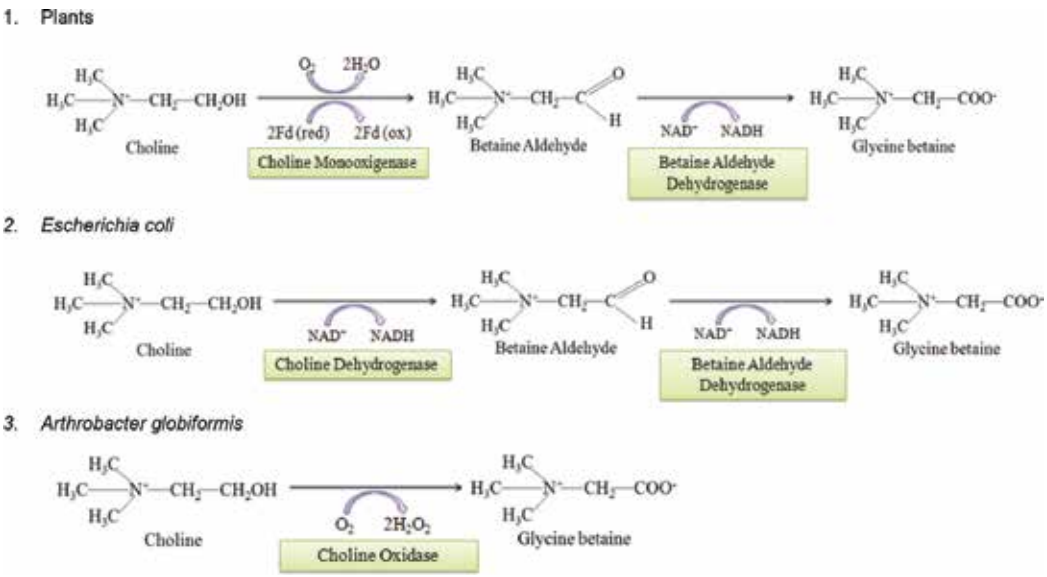


**Figure 3.** The biosynthesis pathway of glycine betaine (GB) in microorganism and plant cells. Choline is oxidized to GB by two enzymes, choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) in plant cells. Depending on the species of microorganism, they operate two distinct pathways. In *Escherichia coli*, choline is oxidized to GB by choline dehydrogenase (CDH) and BADH, but in *Arthrobacter globiformis* choline is converted to GB by single enzyme of choline oxidase (COD).

and maize (*Zea mays*) [97, 98]. The strategy to enhance glycine betaine contents in transgenic plant can be achieved by introduction of the relevant gene under transcriptional control of strong DNA promoter to ensure high-level expression. The genetic engineering of drought-tolerant sugarcane, an important crop for sugar production in tropical areas, with overexpression of the *bet*A gene encoding for CDH from *E. coli* under control of 35S CaMV promoter will be discussed.

Genetic engineering of GB synthesis in plants has focused only with individual gene involved in the pathway of GB biosynthesis. The gene encoding COD from *Arthrobacter globiformis* has been overexpressed in chloroplast of *Arabidopsis* and accumulated in low level of GB [99, 100]. Similar results were obtained with low accumulation level of GB, when the COD was overexpressed in the chloroplast of transgenic rice [101] and COX was expressed in the chloroplast of three diverse species, *Arabidopsis*, *Brassica napus*, and *Nicotiana tabacum* (tabacco) [102]. In addition, the expression of CMO which is catalyzed oxidation of choline in plant cell, accumulated very low GB content in the chloroplast of transgenic tobacco [103] and transgenic rice [104]. The low level of GB in the transgenic plant due to limitation of choline contents in the site targeted synthesis of GB, where the synthesis of choline occurred in cytosol. When COD is targeted to express in the cytosol, the synthesis of GB was 3–5 fold greater when the enzyme was targeted to chloroplast [34]. Substantial increase of GB level was obtained when the transgenic plants expressing COX in their chloroplast feed with choline [102] and in transgenic tobacco expressing chloroplast CMO were supplied with choline or phosphocholine [103]. The GB accumulation in transgenic plants is affected by choline availability, type of transgene, and promoter type [87]. Availability of choline as the substrate for GB synthesis either exogenous or endogenous supply

can enhance GB accumulation in transgenic plants. The GB accumulation that depend on the type of transgene and that of microorganism-derived gene for the pathway of GB synthesis is more potential, and constructing targeted gene under strong promoter is preferable. Although the GB was accumulated at low level, the transgenic plants expressing COD or COX showed enhanced environmental-stress tolerance and had better growth [34, 87].

Plant tolerance environmental stress should be achieved by genetic engineering of BADH since the enzyme acts oxidizing betaine aldehyde into GB. The betaine aldehyde is a toxic compound and should not be accumulated in the cell. Transgenic carrot (*Daucus carota*) expressing BADH in the chloroplast grew well in the presence of NaCl up to 400 mM, whereas control non-transgenic plant showed severe growth retardation [105]. BADH enzyme activity, GB contents, and growth rate were enhanced under salt stress 100 mM NaCl in transgenic carrot compared to the control non-transgenic. Expression of cDNA for BADH from GB accumulator of spinach and sugar beet targeted in the chloroplast of transgenic tobacco increased the level of BADH activity, but failed to accumulate GB in the absence exogenous supply of betaine aldehyde [106]. Similar result was reported in transgenic tobacco transformed with cDNA for BADH from barley [107]. These results suggest that the increase in expression of BADH alone is not sufficient for the increase of GB in transgenic plant and that BADH might possibly participate in other metabolite pathways [108].

Microorganism CDH is an useful enzyme for enhancing accumulation of GB into new species because the enzyme is able to catalyze in two reactions such as oxidation of choline to betaine aldehyde and also converting betaine aldehyde into GB [90, 109]. The purified recombinant CDH from *Halomonas elongata* showed similar subtract specificity with either choline or betaine aldehyde as the substrate [92]. Although gene encoding for CDH (*bet*A) has been poorly used for genetic engineering, increasing GB content and enhancing salt and drought tolerance have been proven in transgenic plants. Introducing *bet*A gene isolated from *E. coli* resulted in elevation of CDH activity and created salt tolerance phenotype of transgenic tobacco as well as an increase in the biomass dry weight [110]. The measurement of CDH activity in transgenic tobacco plants showed considerably higher CDH activity around 4.5–6 fold when compared with the wild type. In parallel with increasing CDH activity, the growth of transgenic tobacco was enhanced by salt stress at concentration 200–300 Mm NaCl. In addition, the level of endogenous GB was found to correlate with the degree of salt tolerance in maize lines and that the gene conferring GB plays a key role in osmotic adjustment [111]. Therefore, maize that transformed with the *bet*A gene from *E. coli* accumulated higher level of GB and more tolerant to drought stress than wild-type non-transgenic plants [98]. The measurement of CDH activities in transgenic maize showed increasing the activities 3-4 fold compared with non-transgenic maize and resulted in greater GB concentration compared with non-transgenic maize. The GB concentration was only 1.2 umol/g FW (fresh weight), but increased significantly up to 2.6–4.0 umol/g FW in the leaves of transgenic maize. Observation of total soluble sugar, free amino acids, and osmotic potential in the leaves of transgenic maize were not significantly increased, but photosystem II and net photosynthesis of transgenic maize were more stable than that in the control non-transgenic maize. These indicate that enhanced GB content has a beneficial effect on osmotic adjustment in condition of drought stress and protect from the damage due to dehydration. The most importantly, the grain yield of transgenic

maize, overexpressing *bet*A gene from *E. coli*, was significantly higher than that of the control non-transgenic after drought treatments [98]. Similar result is also reported that transgenic cotton (*Gossypium hirsutum* L.) expressing *bet*A gene from *E. coli* enhanced GB accumulation and drought tolerance [112]. The GB concentration in the leaves of transgenic cotton was higher compared to wild-type plants in non-stress condition and the GB concentration was much elevated after drought stress condition reached at 282.3–308.4 umol/g dry weight or around 2.3–2.6 fold compared with wild-type plants. The measurement of osmotic pressure showed that the osmotic adjustment was higher in transgenic than in the wild type plant, and the higher osmotic adjustment was significantly correlated with the GB concentration in drought stress condition. The results indicate that GB plays a more important role in osmotic adjustment and maintains the membrane stability than that in slightly increased of soluble sugar and amino acid contents in transgenic cotton. Moreover, after 4 days drought stress treatments, the wild-type cotton showed starting to wilt and decreased leaf relative water contents, whereas wilting symptom did not observe and the leaf relative water content remained higher until 10 days of the drought treatments in transgenic cotton. As observed in the transgenic maize, the transgenic cotton seed yield is significantly greater than in wild-type non-transgenic cotton under drought stress [112]. All together, these studies imply that engineering of GB content using *bet*A gene for CDH from *E. coli* enhances GB content that acts not only as an osmoprotectant, but also stabilizes the structure and activities of protein-enzymes, and maintains the integrity of membrane against damage caused by the drought stress, which in turn increase growth and productivity of the transgenic plants.

Several experiments on the effect of addition exogenous GB have been reported to improve sprouting sugarcane bud under heat and chilling stresses [113]. However, there is almost no report concerning accumulation of GB content in sugarcane. Therefore, enhancing GB synthesis with genetic engineering is considered to be a potential method for improving drought stress tolerance in sugarcane. The drought-tolerance transgenic sugarcane has been developed by introduction of *betA* gene encoding for CDH from *Rhizobium meliloti* (Australian Patent Office, Patent No. 737600 – Inventor(s); Naoki Katsurada, Tsushi Hayakawa, Haruhumi Miwa). The *bet*A gene was constructed in binary vector under the control of strong promoter CaMV35S by Ajinomoto Co., Inc., Japan and used for sugarcane transformation. The *Agrobacterium*-mediated transformation was conducted using explant from BL sugarcane cultivars by the state-run sugarcane producer PT Perkebunan Nusantara XI Indonesia in collaboration with Ajinomoto company and University of Jember. After screening of transformed plantlet in selection media containing hygromycin antibiotic, the resulted plantlet was acclimated in green house and used for analysis. The characterization of the transgenic sugarcane was carried out by analysis of the plants grown in greenhouse experiments.

Genomic analysis by PCR (polymerase chain reaction) and Southern Blot confirmed the presence of stable insertion of *bet*A in the genome of the transgenic sugarcane. As expected, the transgenic sugarcane lines appeared to contain a low-copy insertion of *bet*A gene, whereas non-transgenic plant almost had no DNA hybridization. The stable integration of *bet*A gene was confirmed by PCR analysis in third generation of transgenic sugarcane after vegetative propagation. Although the expressions of gene *bet*A at transcriptional and translational levels were not examined, GB contents in the transgenic sugarcane were detected using HPLC

with Inertsil ODS-3 column. The GB content highly elevated in the leaves of transgenic sugarcane ranged 182–880 ppm, but almost not detected in the control non-transgenic sugarcane after drought condition. The increasing of GB contents sugarcane enhanced drought-tolerance of transgenic sugarcane. Observation of plant morphology during exposure to drought stress by stop watering showed that non-transgenic sugarcane started to wilt at 8 days and permanently wilt at 28 days after drought stress. However, the transgenic sugarcane still vigorously growth at 8 days and start to wilt at 12 days, and then permanently wilt after more than 30 days of drought stress treatments (**Figure 4A, B**). Moreover, expressing gene *betA* also induced salt-tolerance of the transgenic sugarcane. When cultured in media containing 200 mM NaCl for 3 weeks, the drought-tolerance sugarcane showed stay-green, but the wilt-type leaves were yellowish and partly dried. Interestingly, observation of the root profile of the transgenic sugarcane showed a wider and longer root system compared to the wild-type sugarcane, but there was no change in the appearance of the shoot morphology (**Figure 4C**). The improved root system has a good water absorption system to extract limited water availability from deep soil and this is a criterion of drought-tolerance sugarcane [114, 115]. These results imply that the enhanced GB contents in transgenic sugarcane provides an osmoprotectant, stabilizes the structure of macromolecule, maintains the integrity and proper function of the cell membranes, and helps the sugarcane acclimate to drought- and salt-stress condition.

To investigate the growth and productivity of transgenic sugarcane under water limited condition, the sugarcane was grown in non-irrigated dry land of experiment station. Cultivation of the transgenic sugarcane was carried out under confined and limited field trial system according to the regulation for assessment of genetically modified organisms (GMO). Comparison of the drought-tolerance transgenic sugarcane with the wild-type showed almost no difference in the germination of lateral buds and the initial growth rate.

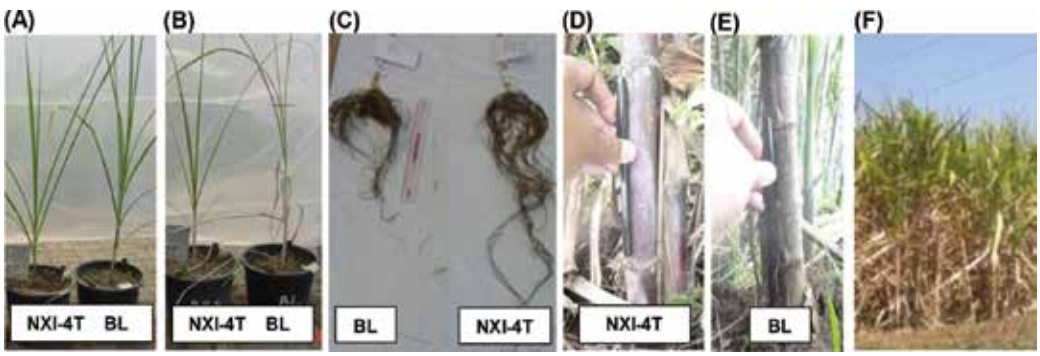


**Figure 4.** Growth performance of drought-tolerant sugarcane overexpressed *betA* gene in greenhouse and in non-irrigated experiment field station using confined trials system. Two-months old NXI-4T (transgenic drought-tolerant) and BL (non-transgenic) sugarcane cultivars were grown in greenhouse and treated with drought stress by stop watering. The transgenic drought-tolerant NXI-4T sugarcane stay green (left) and non-transgenic BL sugarcane cultivars (right) started wilting (A) after 8 days drought stress, then BL sugarcane being permanently wilting and dried after 28 days without watering (B). Wider and longer root profile of NXI-4T (right) is compared to BL (left) sugarcane (C). Normally growth internode of NXI-4T (D) and retarded internode of control BL sugarcane cultivars (E). Growth performance of nine-months old drought-tolerant sugarcane (F). The figures were provided by Dr. Nurmalasari of PT. Perkebunan Nusantara XI, Surabaya, Indonesia.

However, with the start of dry season, non-transgenic sugarcane showed retardation and elongation of stem. The internode of non-transgenic sugarcane becomes shorten during the dry season, but not the internode of transgenic sugarcane (**Figure 4D, E**). The internode of transgenic sugarcane was normally elongated at the similar size as expected and was not affected by dry season. The measurement of sugarcane yield of cane stalk significantly increased compared with non-transgenic plants, although the sucrose content was not different. Similar results were observed that drought-tolerance sugarcane lines have higher productivity stalk height and stalk weight than the susceptible line [116, 117]. In conclusion, all together the results showed that the transgenic sugarcane expression *betA* gene is a drought-tolerant sugarcane and this sugarcane should be the first drought-tolerant sugarcane developed by biotechnology approach.

The Cartagena Protocol on Biosafety (2000), which protects biological diversity from the potentially risk due to the use of transgenic plants, has been ratified by Indonesian government. Thus, for the commercialization of drought-tolerance sugarcane, biosafety assessment has been completed such as environmental safety, food, and feed safety. The environment safety assessment claimed that the drought-tolerance sugarcane has no effect on biodiversity, the occurrence of gene flow, and potentially to be an invasive crop. Bioinformatics BLASTP analysis suggested CDH protein encoded by *bet A* did not have similarity with allergen data base (NCBI Entrez) and potentially to be allergen. Further analysis using animal feeding experiment and simulation digestion system found that the drought-tolerance sugarcane did not potentially toxic or allergen. Based on the biosafety assessment, the drought-tolerance of sugarcane has been approved by the National Genetically Modified Product Biosafety Commission for commercial cultivation in the state-run sugarcane producer PT. Perkebunan Nusantara XI [118]. The company claimed that the drought-tolerance sugarcane produces 10–30% higher sugar productivity under dry land than in conventional parental lines.

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# Fertilization and Harvest

Chapter 9

# Mineral Nutrition and Fertilization of Sugarcane

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Additional information is available at the end of the chapter

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**Abstract**

Sugarcane extracts large amounts of nutrients from the soil and accumulates them in the plant due to its large mass production. Thus, agricultural practices ensuring adequate supply of nutrients to the crop must be adopted to obtain high crop yields in the cane plant cycle and small decreases in the subsequent cycles. In this chapter, the following items will be addressed and discussed: soil sampling, soil fertility evaluation, liming, plastering, cane plant chemical fertilization, sprout chemical fertilization, sugarcane nutritional status evaluation, organic fertilization, use of cultural remains and residues from sugar and alcohol industry, use of humic substances, fertilization, and quality of the sugarcane broth.

**Keywords:** production system, liming, mineral fertilization, nutritional status, green manure, crop residues

## 1. Introduction

Sugarcane is a crop adapted to tropical and subtropical climates, developing well between 37° N in southern Spain and 31° S in the Republic of South Africa. It is planted at altitudes ranging from sea level up to 1.00 m. In addition to the production of sugar and alcohol, sugarcane has been widely used by small and medium-sized rural producers for the production of *cachaça*, *rapadura* (raw brown sugar) and brown sugar, as well as for the feeding of ruminants and pigs, especially during times of high purchase price of corn or of low sale value of this monogastric. In order to increase the productivity of inputs, land and agriculture, agricultural techniques have been adopted, among which we may mention the improvement of soil

physical and chemical properties by the application of lime and gypsum, chemical fertilization, green fertilization, and use of organic compounds. The choice of sugarcane varieties with a greater productive potential is another technology adopted by producers. For this, it is recommended to consult local or regional research agencies, as well as sugar mills and distilleries, to seek information on the adaptation and productivity of sugarcane varieties in different environments and different cultural managements [1].

The average yields of sugarcane, including dry leaves and buds, oscillated around 100 tons of natural matter per hectare. However, by planting improved varieties and correcting and maintaining soil fertility by applying lime, gypsum and fertilization, it is possible to reach productivities of more than 150 tons of natural material per hectare. Complementary irrigation, especially that performed after sugarcane cutting, has resulted in high productivities and greater longevity of sugarcane plantations, as verified by authors in studies conducted in Paracatu, northwest of Minas Gerais, where they obtained an average productivity in two cuts of over 200 tons of industrializable culms per hectare per year [1].

In order for sugarcane to have high stalk yields in the plant cane cycle and small decreases in ratoon yields, it is necessary to implement measures to maintain or increase soil fertility. Based on that, the present chapter aims to discuss the main technologies, related to soil fertility and mineral nutrition of plants, used for sugarcane production.

## 2. Nutritional efficiency

Research has shown that there is a difference among sugarcane varieties in terms of efficiency in the absorption and use of nutrients. There are materials presenting a reasonable production even under conditions of low availability of such nutrients in the soil solution, while other varieties, at times more productive, are consequently more demanding. In the analysis of nutritional efficiency of a variety of sugarcane, its capacity to absorb and use nutrients for the production of dry biomass, protein and sucrose is quantified. The variety that, in the same soil and climatic conditions, accumulates more nutrients is considered more efficient in the absorption process, and the variety that produces a greater mass of sucrose or biomass in relation to mass of an absorbed nutrient is the most efficient in the use of such element [1]. It is desirable that the variety be efficient in both processes, but this is not always achieved.

Currently, there are several sugarcane cultivars with good agronomic, industrial and zootechnical characteristics, such as adaptation to different edaphoclimatic environments, erect growth and resistance to falling, which facilitates harvesting, high yield of culms and sucrose, vigor of sprouts, tolerance to major pests and diseases, and a good dry matter digestibility. It is recommended to plant more than one variety of sugarcane so that, in case of an eventual break of disease resistance or a sudden problem with the cultivar, production will not be significantly compromised. When working with several varieties, varietal management should be adopted to use the good characteristics of each variety to the maximum. Having defined the varieties to be planted, it is necessary to make sure of the quality of seedlings. They should preferably be chosen from nurseries with a good sanity, ages varying between 9 and 12 months, and first, or at most, second cutting.

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## 3. Evaluation of soil fertility

Sugarcane, because it produces large amounts of mass, consequently extracts and accumulates a great quantity of nutrients from the soil. In studies conducted in Brazil, Australia, India, and Florida, it was found that for a production of 120 tons of natural matter per hectare, corresponding to about 100 tons of industrializable culms, the accumulation of nutrients in plant shoots must be 150, 40, 180, 90, 50, and 40 kg of N, P, K, Ca, Mg, and sulfur, respectively. In the case of the micronutrients iron, manganese, zinc, copper, and boron, the accumulations in shoot biomass, also for a production of 120 t, are around 8.0, 3.0, 0.6, 0.4, and 0.3, respectively [2–4]. **Figure 1** shows the accumulation rate of macronutrients in the shoot biomass of RB867515 planted in February and harvested in July of the following year ("year and a half sugarcane").

Due to the high removal of nutrients by the sugarcane harvest, the nutrient supply capacity of the soil must be known to complement chemical and organic fertilization if necessary and, if there is presence of elements at toxic levels, to reduce its concentration by applying lime and gypsum. Normally, nutrient availability and presence of elements at toxic levels in the soil are evaluated by chemical soil analysis. The history of the area, especially fertilizations carried out, and whether or not there were symptoms of deficiency or of toxicity in previous cultures are also of great value [1, 2].

Usually, soil samples are collected from the layers 0–20 and 20–40 cm. The results of the analysis of the layer 0–20 cm will be used to calculate fertilization and liming, and the results of the layer 20–40 cm may be used for calculations of gypsum needed. In the traditional soil sampling system, the area is divided into homogeneous units, taking into account, among others, the history of the area, soil types (color, texture and depth), location and topography (lowlands, slope and plateau), vegetation cover, and previous fertilizations. The most commonly used instruments for collecting soil samples are augers and cutting blades, also known as straight blades. The use of augers in replacement for straight blades has the advantage of a greater speed in collecting

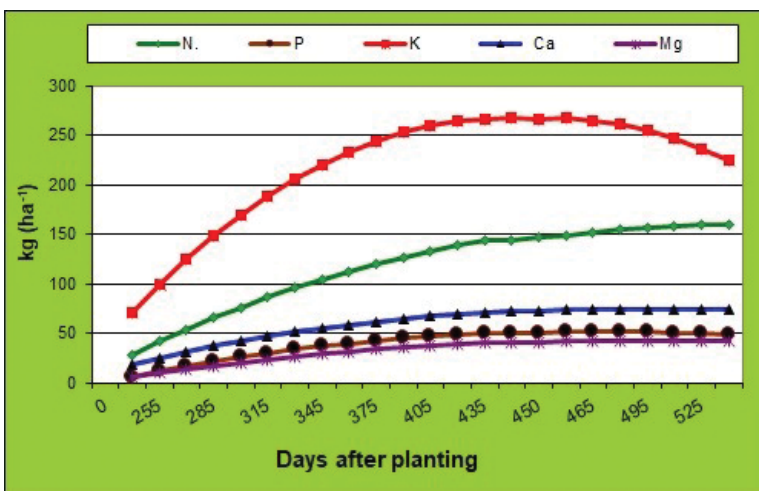


**Figure 1.** Rate of nutrient accumulation in the shoot biomass of RB67515 planted in February and harvested in July of the following year ("year and a half sugarcane").

simple samples, in handling and transporting a small soil volume in field before homogenization of simple samples, and in collecting composite samples. On the other hand, a low volume of collected soil causes variability of soil fertility indexes to increase, making it necessary to collect a high number of simple samples to form a representative composite sample. Even so, the laboriousness of soil sampling using augers is less than when using straight blades. At first, the use of instruments that collect a small soil volume, such as augers, would not be recommended for areas of minimal or no-tillage, where fertilization is performed in planting lines, preferring in such cases straight blades [1]. Regardless of the material used for sampling, care should be taken to always remove the same soil volume from each single sample.

In large areas, grid soil sampling has been used. This technique consists in the collection of georeferenced soil samples. Due to georeferencing, it is possible to measure the variability of soil nutrient contents and to apply acid and fertilizer correctives at variable levels. In the traditional collection system, to obtain a composite sample, one must collect between 10 and 30 simple samples, numbers that depend on the size of the area and its homogeneity. On average, five simple samples per hectare are collected. After air-drying the composite sample, approximately 500 g of soil is collected to be packed in a properly identified container and sent to a chemical analysis laboratory.

In Brazil, potassium, calcium, magnesium, sodium, and aluminum are analyzed as for exchangeable contents, and even though there is a great variation in the chemical extractors used by different laboratories, the accuracy of such analyses is high. Phosphorus, however, presents a greater reactivity with the soil, and its dynamics is also more complex. Thus, there are questions about the results of analyses performed in laboratories using different methods and extractors. However, analyses carried out by authors on soils from sugarcane regions in the state of Minas Gerais, Brazil, not fertilized with natural phosphate, indicated that there was no significant difference between available phosphorus levels extracted using Mehlich in relation to levels obtained using ion exchange resin. Sulfur and micronutrient contents varied greatly in relation to method and extractor used in soil chemical analysis, and there is still a great influence of collection time, soil moisture, and sample preparation [5]. Thus, the history of the area is of great value, especially regarding micronutrients, because if there is a record of deficiency in previous crops, it becomes necessary to include such deficient elements in fertilization.

## 4. Liming

Most soils cultivated with sugarcane in the world are acidic, presenting a low saturation by basic cations such as calcium, magnesium, and potassium. Deficiency of basic cations, associated with high levels of aluminum, iron, and manganese, is detrimental to the growth of the root system and, consequently, of the sugarcane plant as a whole. $Al(OH)_2^+$ and $Al^{3+}$ are phytotoxic forms of aluminum that affect cell division, inhibit root growth, cause phosphorus precipitation both in the soil and inside roots, decrease the absorption of water and nutrients, and affect photosynthesis and, consequently, crop productivity. After applying limestone, there is an increase in the soil pH, and a neutralization of soil acidity precipitates aluminum and makes phosphorus available. Studies conducted by [6] on Purple Latosol showed that a

pH increase from 4.0 to 5.0 precipitates aluminum totally and raises the phosphorus content from 4.8 to 24.2 mg/dm$^3$ (**Table 1**).

The use of nitrogen fertilizers, mainly ammoniacal, and the removal of basic cations by harvesting may also contribute to soil acidity, which is why it has been common practice in sugarcane crops to correct soil acidity. Acidification caused by an ammoniacal fertilizer, ammonium sulfate, $(NH_4)_2SO_4$, is exemplified below:

$$(NH_4)_2SO_4 \leftrightarrow 2NH_4^+ + SO_4^{2-} \quad (1)$$

then $2NH_4^+$ originating from the dissociation of $(NH_4)_2SO_4$ is oxidized by Nitrosomonas and Nitrobacter, producing $2NO_3^-$. Thus, the acid reaction of ammonium sulfate can be described as:

$$(NH_4)_2SO_4 + 4O_2 \rightarrow 2NO_3^- + SO_4^- + 2H_2O + 4H^+ \quad (2)$$

Since 100 g of $CaCO_3$ neutralizes 2.0 moles of $2H^+$, to neutralize $4H^+$, 200 g of $CaCO_3$ is required. Several materials can be used as soil acidity correctors. The most used are calcitic limestones, magnesium and dolomitic limestones, and calcium and magnesium silicates, called steel plant slags. In these slags, the magnesium oxide content oscillates around 8%, while calcitic limestones have MgO contents lower than 5%, magnesium levels between 6 and 12%, and dolomitic levels above 12%. The efficiency of these products in the correction of soil acidity depends, among other factors, on their particle size, a uniform distribution in the field, and soil water availability. In relation to the corrective dose, there are some methods to estimate the quantity of product to be applied. Such methods are based on the particle size and neutralizing power of the corrective, as well as soil chemical characteristics, mainly calcium, magnesium, potassium, aluminum, and hydrogen contents.

In the majority of Brazilian states, the corrective dose to be applied is estimated by neutralization of exchangeable acidity and increase in calcium and magnesium contents [7], or base saturation [8]. For sugarcane, it has been recommended to increase base saturation (V) to 60%. According to [8], the amount of limestone (QC) to be used, when adopting the base saturation criterion, is calculated by the following expression:

$$QC\ (t\ ha^{-1}) = [(60–V) \times T]/PRNT \quad (3)$$

| pH $CaCl_2$ | Ca | Mg | K | $Al^{+3}$ | (H + Al) | P |
|---|---|---|---|---|---|---|
| | (cmolc dm$^{-3}$) | | | | | (mg dm$^{-3}$) |
| 4.0 | 1.80 | 0.66 | 0.37 | 1.60 | 12.56 | 4.8 |
| 4.5 | 4.40 | 0.68 | 0.38 | 1.00 | 10.00 | 5.5 |
| 5.0 | 7.6 | 0.70 | 0.35 | 0.00 | 6.73 | 24.2 |
| 6.0 | 10.60 | 0.70 | 0.36 | 0.00 | 3.66 | 16.0 |
| 7.0 | 15.00 | 0.66 | 0.36 | 0.00 | 0.20 | 8.0 |

Source: adapted from [6].

**Table 1.** Neutralization of soil acidity using $CaCO_3$, increase in pH, precipitation of aluminum, and availability of phosphorus from a Purple Latosol.

where V is the current base saturation of the soil, T is the cation exchange capacity at pH 7.0, and PRNT is the relative total neutralizing power of the corrective used.

Studies conducted by [9] on soils cultivated with sugarcane in the Minas Gerais state showed a need to use twice as many corrective levels as calculated using both methods [7, 8] to neutralize exchangeable aluminum or increase base saturation to 60% (**Figure 2**). Results similar to those described by [9] were obtained by [10–12] by comparing analytical methods to assess the need for limestone in the states of Santa Catarina, Paraná, and Mato Grosso. The authors also verified that base saturation underestimated at a high degree the need for limestone by the soils studied, especially the most buffered ones. Base saturation values lower than those predicted analytically were also found by [13] in a medium texture, alkaline Latosol cultivated with sugarcane. Ref. [12] in Campo Novo do Parecis and Nova Mutum (MT) verified that the increase in limestone doses estimated by base saturation ranged from 46 to 92%. Considering the observations of [10–13], the authors recommended that, for areas with base saturation values below 30% or more clayey soils, the amount of limestone to be applied is 1.5–2.0 times as that calculated by Eq. (3) [8].

In large sugarcane crops, many types of limestone distributors have been used, but, for small producers, the application is manual for most of the time. One method that authors have recommended for small producers is to demarcate a square or a rectangle with the limestone itself and, in this area, apply a corrective volume corresponding to the recommended dose. For example, supposing that the recommended dose was 4000 kg and the density of this correction is 1.25 kg/L, then 3200 L of corrective should be applied per hectare, or 0.32 L of the corrective/$m^2$. One of the options for the producer to manually distribute limestone would be to demarcate 50 $m^2$ areas with the limestone itself and apply 12.8 L of limestone. In **Figure 3**, a small sugarcane producer is applying limestone using this method to demarcate an area. Two bamboo sticks, spaced 10 m apart, can be seen at the bottom, with a plastic tape tied at the edge to serve as a marking for the demarcation of lines.

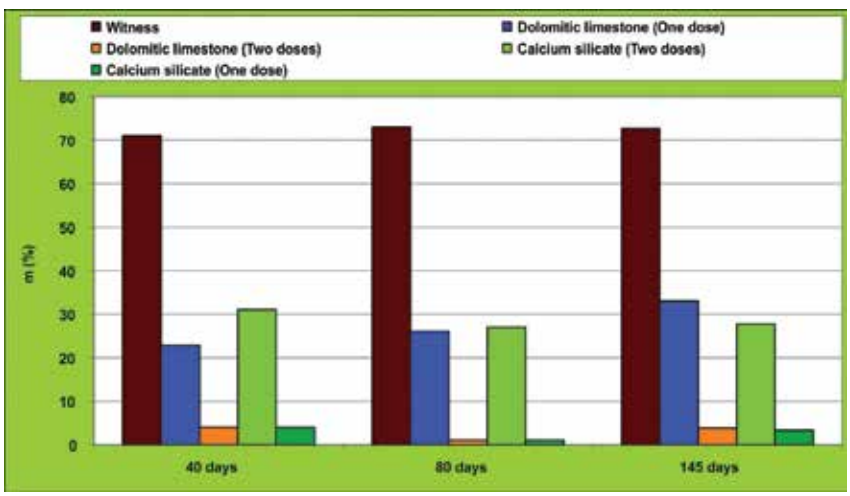


**Figure 2.** Aluminum saturation (m%) at 40, 80, and 145 days after the beginning of incubation (DAI) of soil samples with dolomitic limestone and calcium silicate using one or two doses of corrective analytically predicted by base saturation.

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**Figure 3.** Equipment for the distribution of limestone in large sugarcane plantations, and a small rural producer applying limestone to previously demarcated areas.

There is a generalized conceptualization that the best $Ca^{+2}$:$Mg^{+2}$ ratio in the soil is 4:1. Therefore, the type of limestone (calcitic, magnesian, or dolomitic) to be used should be based on this ratio. On the other hand, some authors recommend exchangeable cation saturation in relation to the effective cation exchange capacity of the soil (t) at 80% of calcium, 13% of magnesium, and 6% of potassium, providing Ca:Mg, Ca:K, and Mg:K ratios of 6.15:1, 13.3:1, and 2.2:1, respectively. However, several studies have shown that the concentrations of Ca and Mg in the solution are more important than the relation between these cations [14]. In the case of corn, studies conducted by [14] indicated that variations in the soil Ca:Mg ratio from 1:1 to 12:1 in soils with exchangeable Ca and Mg contents above 2.32 and 0.40 $cmol_c\ dm^{-3}$, respectively, did not affect yield and production of corn dry matter.

The sugarcane plantation areas and sugarcane planting using minimum and no-tillage systems have increased, following the tendency of corn and soybeans. In these systems, limestone is not incorporated as in the conventional tillage. However, the mineralization of crop remains and sugarcane straw, similar to what occurs in no-tillage areas with annual crops, releases organic anions that complex with Ca, Mg, K, and Al, forming electrically neutral molecules that percolate in the soil. In addition, such organic anions neutralize part of the soil acidity. Therefore, in such areas, liming should be performed only when base saturation at the 0–20 cm layer is lower than 40%.

In a study conducted by [3] using lysimeters, it was verified that the sum of cation charges (K, Ca, Mg, and Na) was always greater than the sum of anion charges (nitrate, sulfate, and chloride) for the whole experimental period. Sulfate was the mineral anion with the highest concentration in the solution percolated in the soil, followed by chloride and nitrate. Initially, organic anions represented only 40% of the total negative charge, but there was a gradual and constant increase of these anions in the ionic balance of the percolated solution and, at the end of the experimental period, their share of the solution's electroneutrality increased to 70%. Such results confirm, as in other studies, that organic anions originating from the mineralization of sugarcane remains or released by sugarcane roots must be involved in the nutrient leaching process by organometallic complexation with Ca, Mg, K, Al, and Na, which are present in the soil solution.

## 5. Gypsum

Agricultural gypsum, $10CaSO_4.2H_2O$, a by-product of the fertilizer industry, originates from the reaction between sulfuric acid and phosphate rocks used to produce phosphoric acid. Gypsum applied to soil does not neutralize soil acidity but decreases aluminum saturation and increases base saturation of the subsurface, providing conditions for a further development and deepening of the sugarcane root system. It is recommended to apply gypsum when $CaC^{2+}$ contents are lower than 0.4 $cmol_c\ dm^{-3}$ and/or aluminum saturation is greater than 20% at the 20–40 cm layer. The application of gypsum will lead to the improvement of the root environment at layers below arable ones, an effect that lasts for several years. For this reason, the annual reapplication of gypsum is not necessary. In areas with sugarcane straw or organic residues on the soil, and if the contents of $Ca^{2+}$ are not very low and/or aluminum saturation is not very high, the response to gypsum may be lower.

The doses of gypsum to be applied may be based on the need for liming, or on soil texture. The amount of gypsum to be applied varied between 25 and 30% for the need for liming, multiplied by a depth correction factor (profile to be corrected/20). For example, the amount of limestone to be applied was 3.0 t $ha^{-1}$, and improvement of the root environment at the 20–60 cm layer is desired. Then, the amount of gypsum will be equal to 1.5 t $ha^{-1}$[(3.0 x 0.25) x (60–20)/20]. When the doses of gypsum to be applied are based on soil texture, the following recommendation can be used [8]: dose to be applied (kg $ha^{-1}$) = clay (g $kg^{-1}$) x 6.0.

Gypsum is applied in total area and may or may not be incorporated into the soil. When it is not possible to use it, mainly because of difficulty in acquiring it in small quantities, a fact that usually happens with micro and small farmers, one should choose to apply simple superphosphate as a source of phosphorus because this fertilizer contains calcium sulfate. In a study conducted by [15], limestone and gypsum rates were studied in a sugarcane crop cultivated in medium texture soils with a low cation exchange capacity. A relation between calcium levels in the soil and growth of the root system was also observed. Twenty-seven months after the beginning of the study, in a treatment with the application of 2.8 t of gypsum per hectare, the highest yield of biomass and industrializable shoots occurred. By soil

| Layer (cm) | Exchangeable calcium ($cmol_c\ dm^{-3}$) | Root mass (g $dm^{-3}$) | % of root system |
|---|---|---|---|
| 0–25 | 2.10 | 4.4 | 29.93 |
| 26–50 | 1.37 | 3.0 | 20.41 |
| 51–75 | 0.90 | 2.4 | 16.33 |
| 76–100 | 0.82 | 2.0 | 13.61 |
| 101–125 | 0.70 | 1.8 | 12.24 |
| 126–150 | 0.60 | 1.1 | 7.48 |

Source: adapted from [15].

**Table 2.** Calcium content in the soil and growth of sugarcane root system in a soil that received limestone and gypsum.

analysis, a relation between exchangeable calcium and sugarcane root system was found: at 150 cm depth, $Ca^{2+}$ was 0.60 $cmol_c/dm^3$ and the root mass was 1.1 $g/dm^3$. Several authors have reported that under conditions of low availability of calcium in the soil, sugarcane roots concentrated at the layer 0–30 cm. However, in this study, 50% of the root system mass was in the layer 51–150 cm (**Table 2**).

## 6. Liming in sugarcane regrowth areas

Soil calcium and magnesium contents decrease during sugarcane cycles both by the removal of bases by harvests and by acidification caused by nitrogenous fertilizers. This effect is demonstrated in the long-term study (**Figure 4**) conducted by [15, 16]. These authors evaluated the reacidification of a soil cultivated with sugarcane by five cuts.

Initially, the soil presented, at the layers 0–20 and 20–50 cm, a base saturation of 15 and 7%, respectively. At the time of preparation of the soil for planting sugarcane, 2.5 t of limestone and 1.5 t of gypsum were applied per hectare. Soil chemical changes in plant cane and regrowth are shown in **Figure 4**. After plant cane thinning, base saturation at the layers 0–20 and 20–50 cm was, respectively, 52 and 38%; by the fifth cut, the values were similar to those observed at the time of reforestation.

The authors of this chapter have recommended liming for regrowth areas when there is a base saturation of less than 50% at the 0–20 cm layer. The application of corrective should be in the total area preceding crop treatments and calculating the necessary amount as previously described.

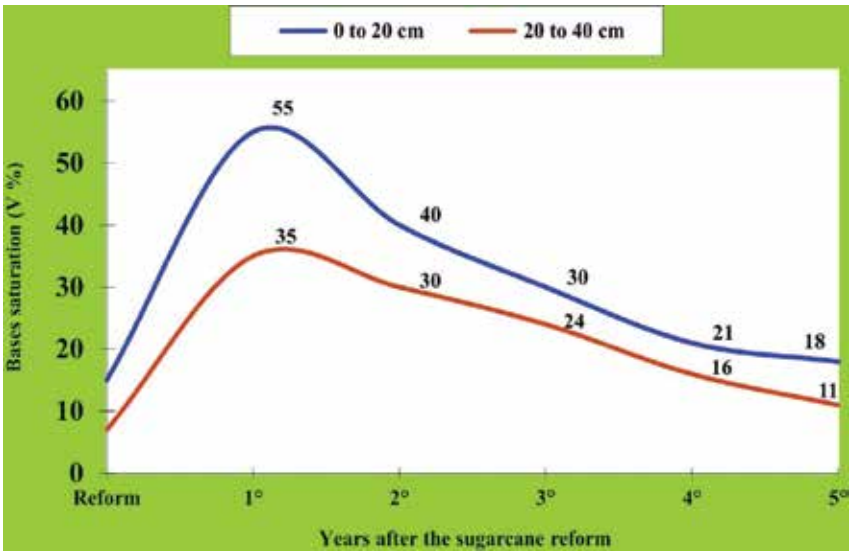


**Figure 4.** Changes in the base saturation of a soil cultivated with sugarcane. Source: adapted from [15, 16].

## 7. Mineral fertilization

The mineral fertilization of sugarcane is based on the results of soil analysis at the 0–20 cm layer and on the productivity desired.

### 7.1. Nitrogen in plant cane

Nitrogen is important for the nutrition and physiology of sugarcane because, among other functions, it is a constituent of all amino acids, proteins, enzymes, and nucleic acids [17]. Nitrogen and potassium are absorbed in greater amounts by this crop [3]. The absorbed nitrogen increases the meristematic activity of shoots, resulting in greater tillering and leaf area index (LAI). Furthermore, N increases leaf longevity. Such an increase in LAI increases the efficiency of use of solar radiation, measured as the fixation rate of carbon dioxide (μmol of $CO_2$ $m^{-2}$ $s^{-1}$), thus increasing accumulation of dry matter.

The accumulation of nitrogen by sugarcane varies according to cultivar, crop age, and availability of N and other elements in the soil solution and also depends on soil and climatic factors. For the more common varieties planted, nitrogen extraction ranges around 1.2 kg per ton of natural matter of shoots. Considering that roots and rhizomes correspond, on average, to 30% of the mass of the whole plant, it can be estimated that for each t of natural matter accumulated by shoots, there is an absorption of 1.5 kg of N by the plant. Therefore, for systems with a productivity greater than 120 tons of natural matter per hectare, the amount of N absorbed by the crop exceeds 180 kg $ha^{-1}$. In these systems, the use of nitrogen fertilization at doses ranging from 60 to 100 kg $ha^{-1}$ is suggested [1].

Nitrogen uptake and nitrogen metabolism are greatly influenced by phosphorus availability. In plants with inadequate phosphorus supply, there is a decrease in the nitrate absorption of the soil solution. The nitrate translocation from roots to shoots decreases, thus increasing the accumulation of amino acids in leaves and roots. Ref. [18] observed an enormous influence of the availability of P, both of nutrient and endogenous solution, on corn nitrogen uptake and metabolism (**Figure 4**). Well-supplied phosphorus plants before and during a kinetic study (+P; +P) showed a practically constant nitrate absorption during the experiment. However, plants deprived of P before and during the experimental phase (−P; −P) were unable to absorb the nitrate from the solution.

It is believed that plant cane, because it has a higher phosphorus supply when compared to regrowth, behaves similar to corn plants well supplied with phosphorus (+P; +P). In studies conducted by the authors in the region of Passos, southern Minas Gerais, it was verified that the increase in the dose of phosphorus applied to planting grooves affected larger accumulations of N in the biomass of plant cane, since for each kg of P applied there was an increase of about 1 kg of N. These results are certainly the effects of changes caused in the absorption and metabolism of nitrogen, as observed by [18].

It should be noted, however, that some studies reported a low response of plant cane to nitrogen fertilization, and the causes of such low responses are not sufficiently explained. Several authors have attributed it to experimental variability, to mineralization of organic matter and of crop remains, to fertilizer application times, and to losses by leaching and denitrification

[19, 20]. However, in an experiment conducted by [3] with plant cane cultivated in a sandy soil and fertilized with marked urea ($^{15}N$), losses were not observed with the leaching of nitrogen from the fertilizer (**Figure 5**). The movement of the $^{15}N$-fertilizer was small. More than 70% of the fertilizer recovered in the soil was at the 0–30 cm layer. There was a measurable loss of N native from the soil, or of crop remains, equivalent to 4.5 kg $ha^{-1}$ [3]. Thus, if nitrogen fertilization is applied to plant cane, nitrogen fertilizer, at doses ranging from 60 to 100 kg $ha^{-1}$, should be applied to the bottom of planting grooves along with phosphorus and potassium.

### 7.2. Nitrogen in regrowth

The responses of sugarcane regrowth to nitrogen fertilization are more frequent than in plant cane, with a percentage above 90%. As a general recommendation, it is suggested to apply 1.0 kg of N per ton of natural matter accumulated in shoots. Since industrializable culms represent on average 80% of the natural matter of shoots, yields of 100 t of culms would correspond to 125 t of natural matter. In this case, the recommendation for fertilization would be 125 kg of N $ha^{-1}$, and the nitrogen fertilizer should be applied in a single dose together with potassium.

Urea has been the most used nitrogen fertilizer for sugarcane fertilization mainly because of its lower cost per unit of N compared to other sources. The application of urea to the soil or straw may lead to large losses due to the volatilization of ammonia (approximately 40%) [1]. Therefore, it is recommended to bury it into the soil at a depth of approximately 7.0 cm. When it is not possible to bury the urea in the soil, it must be irrigated to incorporate it into the soil or to fertilize it before a rain, which is possible only in small areas. If it is not possible to bury

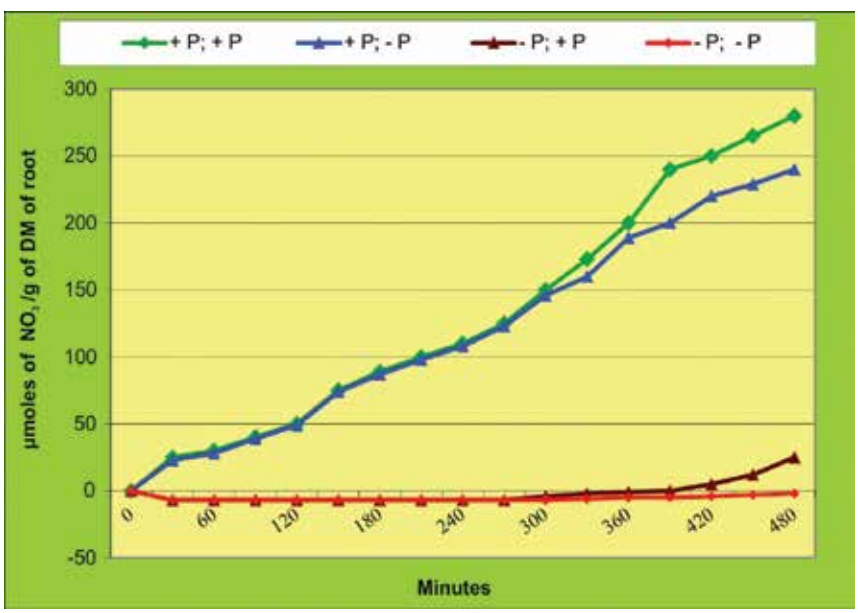


**Figure 5.** Nitrate uptake by corn plants with different phosphorus supplies: adequate before and during the study (+P; +P), adequate before and absent during the study (+P; −P), absent before and adequate during the study (−P; +P), and absent before and during the study (−P; −P). Source: adapted from [18].

urea in the soil, irrigate it, or fertilize it before a rain, one should choose ammoniacal sources, such as ammonium sulfate, or nitric sources.

### 7.3. Phosphorus

The highest dose of phosphorus should be applied to the bottom of planting grooves. Such application at a greater depth increases the nutrient uptake by sugarcane, since water availability at the subsurface varies less than on the surface. The mobility of phosphorus in the soil is small, and its diffusion is influenced by several factors, especially precipitation by cations such as iron, aluminum, and calcium; volumetric content of water in the soil; adsorption of phosphorus by soil colloids; complexity of the environment structure; soil compaction; distance to reach roots; and contents of elements in soil [21]. In general, very low values are recorded for transport of phosphorus due to its strong interaction with soil colloids, especially in very weathered soils. According to [21], it can be estimated that the transport is on average 0.013 mm per day.

Even applying a higher dose of phosphorus during planting, there is a need for phosphate fertilization for regrowth. **Tables 3–5** present recommendations for phosphate fertilization of plant cane at the bottom of planting grooves, considering the extractor used in the soil chemical analysis, Mehlich or ion exchange resin, as well as soil fertility classes.

According to some authors, it is unlikely to obtain a productivity above 150 t when the phosphorus extracted with resin is lower than 6.0 mg $dm^{-3}$. However, in studies conducted in newly developed Cerrado areas in the northwest of Minas Gerais on a phosphorus content lower than 6.0 mg $dm^{-3}$, yields were higher than 200 tons of culms per hectare in a plant cane with a 14-month cycle fertilized with 100 kg of P per hectare and receiving complementary irrigation of only 120 mm [1].

Phosphorus applied during sugarcane planting ensures, in most cases, an adequate supply of this element to plant cane and the first regrowth. Formulations containing P in the fertilization of later regrowth should be used. Prior to phosphate fertilization, the soil should be analyzed at the 0–20 cm layer and, if the base saturation (V) is less than 50%, it is recommended to perform first a liming to raise the V to 60%. As shown in **Table 1**, the absence of exchangeable aluminum in the soil solution increases the efficiency of phosphate fertilization, especially since there is no formation of aluminum phosphate (a low solubility compound) in the soil

| Clay content (g $kg^{-1}$) | Low | Medium | High |
|---|---|---|---|
| | Available phosphorus classification (mg $dm^{-3}$) | | |
| 0–150 | Less than 20 | 20–30 | Above 30 |
| 150–350 | Less than 15 | 15–20 | Above 20 |
| 350–600 | Less than 10 | 10–15 | Above 15 |
| 600–1000 | Less than 5 | 5–10 | Above 10 |
| | Available potassium classification (mg $dm^{-3}$) | | |
| | Less than 40 | 41 a 90 | Above 90 |

**Table 3.** Soil fertility classes considering clay, phosphorus, and potassium contents extracted with Mehlich.

| Production expectation in the cane plant cycle (t ha$^{-1}$) | Soil fertility class | | |
|---|---|---|---|
| | Low | Medium | High |
| | Dose of P (kg ha$^{-1}$)* | | |
| Less than 100 | 70 | — | — |
| 100–150 | 80 | 60 | 40 |
| 150–180 | 90 | 70 | 50 |
| Above 180 | 100 | 80 | 60 |

*To convert P into $P_2O_5$, multiply the desired value by 2.29.

**Table 4.** Phosphorus doses suggested for sugarcane fertilization based on the availability of phosphorus extracted with Mehlich and on the expectation of natural matter production.

| Production expectation in the cane plant cycle (t ha$^{-1}$) | Extracted phosphorus (mg dm$^{-3}$) | | | |
|---|---|---|---|---|
| | 0–6 | 7–17 | 16–40 | >40 |
| | Dose of P (kg ha$^{-1}$)* | | | |
| Less than 100 | 80 | 44 | 30 | 20 |
| 100–150 | 90 | 55 | 40 | 26 |
| Above 150 | 100 | 66 | 45 | 35 |

*To convert P into $P_2O_5$, multiply the desired value by 2.29.
Source: adapted from [8].

**Table 5.** Phosphorus doses suggested for sugarcane fertilization based on the availability of phosphorus extracted with ion exchange resin and on the expectation of natural matter production.

and within plant roots. If the base saturation is greater than 50% and the P content, extracted with Mehlich, is lower than 10 mg/dm$^3$, a regrowth phosphate fertilization is recommended.

The dose of phosphorus used may be based on the recovery of the P removed by harvesting. In this case, for each ton of natural material, 200–300 g of P should be applied. If, for example, the production of natural regrowth material was 120 t per ha, which corresponds to about 100 t of industrializable culms, from 25 to 40 kg of P should be applied per ha. Phosphate fertilizer should be applied together with N and K. In large crops, regrowth N-P-K fertilization is carried out simultaneously with subsoiling and cultivation of interlines. In small and medium properties, especially those where burnt sugarcane is harvested or produced for animal feed, the furrowing of sugarcane lines using an animal traction plow for later fertilization has presented good results. The N-P-K fertilizer is applied to open grooves in sugarcane interlines and then covered with soil using animal traction.

### 7.4. Potassium

Potassium fertilization of sugarcane is carried out at planting and after each sugarcane cut because potassium is displaced in the soil profile. The mineral fertilization of sugarcane is based on the results of soil analysis at the 0–20 cm layer, on the productivity desired and on the final use of sugarcane. In sugarcane fields intended for cattle feeding, the potassium dose

to be applied should be increased, since nutrient removal will be greater because sugarcane is harvested along with nodes and dry leaves. The amount of potassium contained in nodes and dry leaves of sugarcane ranges around 70 kg per ha [22] and may reach 140 kg per ha in plant cane [3]. **Tables 6–8** present the recommendations of potassium fertilization for plant cane and regrowth, with Mehlich or ion exchange resin as extractors.

The dose of K to be applied to regrowth may be based on the recovery of the potassium removed by the crop, as suggested for nitrogen and phosphate fertilization. This method was adopted by the authors and has been recommended with excellent agronomic and financial results. Although the absorption and the removal of potassium vary among sugarcane cultivars, it can be considered that for each ton of natural matter harvested, there is, on average, a removal of 1.5 kg of K. There is no need to partition the potassium used in regrowth fertilization due to possible losses by leaching. In studies conducted by Oliveira et al. [3] using lysimeters, K losses by leaching were not reported (**Figure 6**). These results were confirmed by [23], who also observed that K losses by percolation below a depth of 100 cm were 9.0 kg $ha^{-1}$, totally compensated by the input of K from rainwater (18 kg $ha^{-1}$).

| **Production expectation in the cane plant cycle (t $ha^{-1}$)** | **Soil fertility class** | | |
|---|---|---|---|
| | **Low** | **Medium** | **High** |
| | **Dose of K (kg $ha^{-1}$)*** | | |
| Less than 90 | 100 | — | — |
| 90–120 | 120 | 100 | 80 |
| 120–150 | 140 | 120 | 100 |
| 150–180 | 160 | 140 | 120 |
| Above 180 | 180 | 160 | 140 |

*To convert K into $K_2O$, multiply the desired value by 1.20. When sugarcane is harvested for animal feed, it is suggested to raise the recommended K dose by 25%.

**Table 6.** Potassium doses suggested for sugarcane fertilization based on the availability of potassium extracted with Mehlich and on the expectation of natural matter production.

| **Production expectation in the cane plant cycle (t $ha^{-1}$)** | **K extracted with resin ($mmol_c$ $dm^{-3}$)** | | | | |
|---|---|---|---|---|---|
| | **0–0.7** | **0.8–1.5** | **1.6–3.0** | **3.1–6.0** | **>6.0** |
| | **Dose of K (kg $ha^{-1}$)*** | | | | |
| Less than 100 | 120 | 100 | 60 | 60 | 0 |
| 100–150 | 160 | 140 | 100 | 80 | 0 |
| Above 150 | 200 | 160 | 120 | 100 | 0 |

*To convert K into $K_2O$, multiply the desired value by 1.20.
Source: adapted from [8].

**Table 7.** Potassium doses suggested for sugarcane fertilization based on the availability of potassium extracted with ion exchange resin and on the expected production.

| Regrowth production expectation (t $ha^{-1}$) | K extracted with resin ($mmol_c$ $dm^{-3}$) | | |
|---|---|---|---|
| | 0–1.5 | 1.6–3.0 | >3.0 |
| | Dose of K (kg $ha^{-1}$)* | | |
| Less than 60 | 90 | 60 | 30 |
| 60–80 | 110 | 80 | 50 |
| 80–100 | 130 | 100 | 70 |
| Above 100 | 150 | 120 | 90 |

*To convert K into $K_2O$, multiply the desired value by 1.20.
Source: adapted from [8].

**Table 8.** Potassium doses suggested for regrowth fertilization based on the availability of potassium extracted with ion exchange resin and on the expected production.

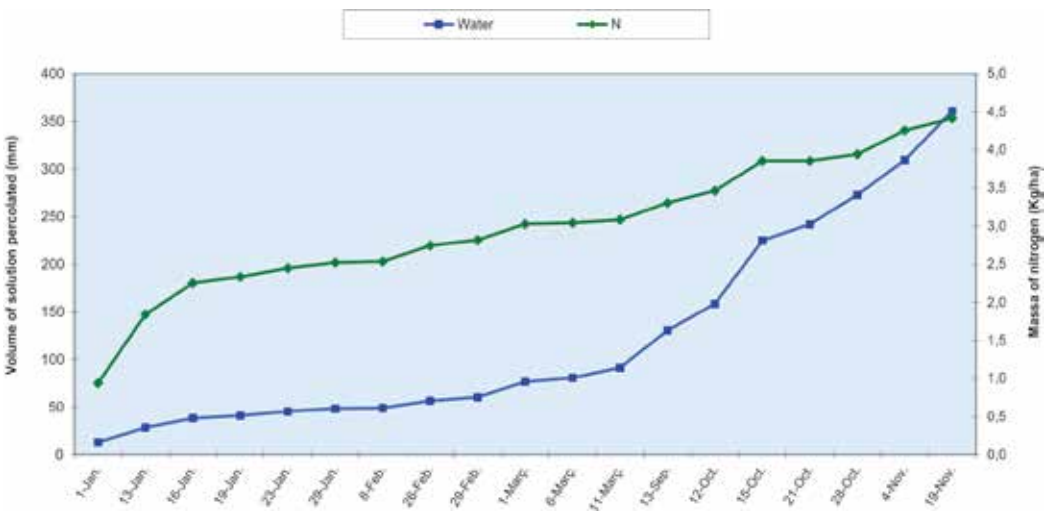


**Figure 6.** Solution volume and mass of percolated nitrogen during the plant cane cycle cultivated in a sandy soil.

Potassium chloride has been the most used source of K in fertilization. However, other residues containing potassium are also used, among them vinasse, a by-product of alcohol manufacture. Vinasse may replace potassium fertilization. Therefore, the amount of potassium supplied by application of vinasse should be fully deducted from mineral fertilization. The volume of vinasse applied ranged from 60 to 300 $m^3$ $ha^{-1}$ depending on the potassium concentration. The concentration of K in vinasse originating from molasses is higher than in others, followed by a mixed must, which contains on average twice as much K as in vinasse originating from sugarcane juice, with values ranging between 2.5 and 1.2 kg $m^{-3}$, respectively (**Table 9**).

### 7.5. Sulfur

Sulfur can be dispensed in areas that received application of vinasse or agricultural gypsum. The critical level of $S\text{-}SO_4^{-2}$ in the soil, extracted with $Ca(H_2PO_4)_2$ 500 mg $L^{-1}$, is 10 mg/dl[3]. In areas in

| Chemical composition | Origin of must | | |
|---|---|---|---|
| | **Molasses** | **Mixed** | **Cane juice** |
| | kg of the element by $m^3$ de vinasse | | |
| N | 0.57–0.79 | 0.33–0.48 | 0.25–0.35 |
| P | 0.05–0.15 | 0.03–0.14 | 0.03–0.07 |
| K | 3.27–6.32 | 1.81–2.78 | 0.95–1.61 |
| Ca | 1.32–1.70 | 0.40–0.95 | 0.08–0.52 |
| Mg | 0.50–0.85 | 0.19–0.35 | 0.13–0.25 |
| S | 0.30–0.40 | 0.45–0.54 | 0.58–0.70 |
| Organic matter | 37.0–57.0 | 19.1–45.1 | 15.3–34.7 |
| | g of the element by $m^3$ de vinasse | | |
| Fe | 52–120 | 47–130 | 45–110 |
| Cu | 3.1–9.3 | 4.2–57.3 | 1.0–18.0 |
| Zn | 3.0–4.0 | 3.0–4.0 | 2.0–3.0 |
| Mn | 6.0–11.0 | 5.0–11.0 | 5.0–10.0 |
| pH | 4.2–4.4 | 3.6–4.4 | 3.5–3.8 |

Source: Analyses carried out by the authors on the vinasse of mills located in Minas Gerais and Alagoas, Brazil.

**Table 9.** Chemical composition of vinasse originating from different musts.

need of this macronutrient, at least 30 kg of sulfur per hectare should be applied using ammonium sulfate or simple superphosphate, which contains, respectively, approximately 210 and 110 g of S per kg of fertilizer (**Figure 7**).

### 7.6. Micronutrients

In most areas cultivated with sugarcane in Brazil, there has been an adequate supply of micronutrients in the soil, thus dispensing their use in chemical fertilizations. However, the implantation of sugarcane plantations in less fertile or marginal areas, associated with fertilization using concentrated fertilizers and the planting of high productivity varieties, which increasingly increase the absorption and export of nutrients, has caused micronutrient deficiency in several sugarcane plantations. In such cases, there is a need for the supply of microelements by fertilization. Soil analysis and area and variety history have been used as predictive methods for assessing the possibility of occurrence of micronutrient deficiency. Soil analysis should be associated to area and variety history since analytical results are influenced by the extractor used, by the characteristics of the soil and of the variety, and also by the time of sample collection. There are reports of marked effects of soil moisture on micronutrient contents [1, 5].

Studies carried out by [24] showed that the best correlations between the Zn or Cu contents in soils and the concentrations of these micronutrients in plants were obtained by the method that uses a solution of diethyl triamine penta-acetic acid (DTPA) as extractor when compared

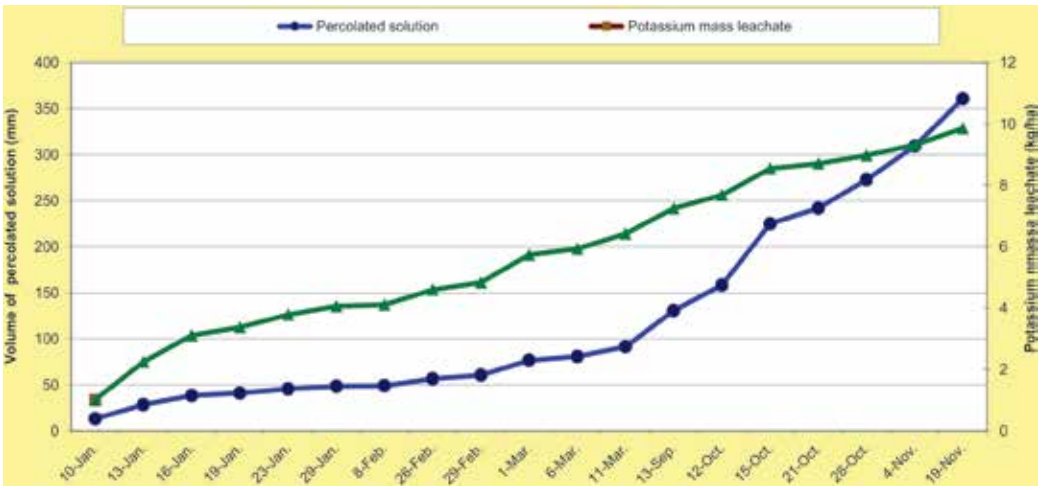


**Figure 7.** Solution volume and mass of percolated potassium during the plant cane cycle cultivated in a sandy soil.

to Mehlich-1 and HCl extractors. According to [24], there is a tendency for DTPA to be more efficient than Mehlich-1 and HCl in situations where the availability of Zn and Cu is changed by liming. As for Mn, acid and chelating solutions have shown very close correlation coefficients between Mn in soil and in plants. However, by analyzing soils fertilized with Mn oxides, there was a tendency of DTPA being the best extractor.

**Table 10** lists the minimum levels of micronutrient availability in soil extracted with DTPA and Mehlich-1 solution, below which such microelements should be supplied to plants by fertilization. The doses of copper, zinc, manganese, and iron to be applied, in case of deficiency, are 2.5–6.0, 5.0–7.0, 3.0–6.0, and 6.0–10.0 kg $ha^{-1}$, respectively, using oxides, chlorides, and sulfates.

In studies conducted by the authors on coastal plain soils in Alagoas, northeastern Brazil, it was verified that even when high-dose manganese and copper sulfates (up to 16.0 kg of element/ha) were applied, RB867515 and RB92579 remained deficient in these elements. The content

| | **Extractor** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **DTPA** | | | | **Mehlich-1** | | | |
| | Element | | | | | | | |
| Available | Cu | Zn | Mn | Fe | Cu | Zn | Mn | Fe |
| | mg $dm^{-3}$ | | | | | | | |
| Low | ≤0.2 | ≤0.5 | ≤1.2 | ≤4 | ≤0.8 | ≤1.0 | ≤6 | ≤19 |
| Medium | 0.3–0.8 | 0.6–1.2 | 1.3–5.0 | 5–12 | 0.8–1.2 | 1.0–1.5 | 6–8 | 19–30 |
| High | >0.8 | >1.2 | >5.0 | >12 | >1.2 | >1.5 | >8 | >30 |

Source: cited by [1].

**Table 10.** Minimum values of micronutrient availability in the soil extracted with a solution of DTPA and Mehlich-1.

of these nutrients in the +3 leaf limbus, used to evaluate nutritional status, was lower than 5.0 and 40.0 mg/kg of dry matter, respectively, for copper and manganese, characterizing a severe deficiency of these elements. The high adsorption of copper and manganese sulfates may have been the cause of the absence of responses. Ref. [25] studied the adsorption of copper originating from several compounds. These authors studied the application of $CuSO_4$ to sandy and humic soils. They found a very high adsorption (99.4%) of copper 2 h after its addition to the soil. On the other hand, copper in the ethylene diaminotetraacetic acid and diaminocyclohexane tetraacetic acid forms presented a soil percentage adsorption of 7.3 and 5.3, respectively. Therefore, it is necessary to evaluate the efficiency of other sources of copper and manganese because the adsorption of copper and manganese sulfates by the soil was very high. In addition to compromising the productive potential of these varieties, copper and manganese deficiency leads to metabolic changes that compromise the quality of the broth. These nutrients are constituents of the polyphenol oxidase and amylase metalloenzymes [17, 26, 27]. Therefore, with a poor performance of these enzymes, there is accumulation of phenolic and starch compounds.

## 8. Evaluation of the nutritional status of sugarcane

The chemical analysis of sugarcane leaves is another way for evaluating the nutritional status of crops. The preference for leaves is because, in general, they reflect better the variations in the supply of nutrients both by the soil and by fertilizations. In sugarcane, it has been recommended to collect the +2 or +3 leaves. The leaf +1 is, in the descending direction of the stem, the first leaf to show a fully visible ligule (region of insertion of the leaf sheath on the stem). For the chemical analysis, the median third of the +2 or +3 leaf is used excluding the central vein.

Samples from the middle third should first be washed in clean running water and then in distilled water. Then, the material should be dried at 65°C until constant weight. If this

| **Authors** | **Nutrient (g kg$^{-1}$)** | | | | | |
|---|---|---|---|---|---|---|
| | **N** | **P** | **K** | **Ca** | **Mg** | **S** |
| [17]* | 19–21 | 2.0–2.4 | 11–13 | 8.0–10 | 2.0–3.0 | 2.5–3.0 |
| [17]** | 20–22 | 1.8–2.0 | 13–15 | 5.0–7.0 | 2.0–2.5 | 2.5–3.0 |
| [28] | 18–25 | 1.5–3.0 | 10–16 | 2.0–8.0 | 1.0–3.0 | 1.5–3.0 |
| [29] | 16–25 | 2.0–3.5 | 6–14 | 4.3–7.6 | 1.1–3.6 | 1.3–2.8 |
| **Authors** | **Nutrient (mg kg$^{-1}$)** | | | | | |
| | **B** | **Cu** | **Fe** | **Mn** | **Mo** | **Zn** |
| [17]* | 15–50 | 8–10 | 200–500 | 100–250 | 0.15–0.30 | 25–50 |
| [17]** | — | 8–10 | 80–150 | 50–125 | — | 25–30 |
| [28] | 10–30 | 6–15 | 40–250 | 25–250 | 0.05–0.20 | 10–50 |
| [29] | 6–29 | 9–17 | 76–392 | 73–249 | — | — |

*Concentration ranges for plant cane.

**Concentration ranges for regrowth.

**Table 11.** Nutrient concentration ranges in the middle third of the +2 or +3 leaf considered adequate.

drying is not possible, the samples should be sent quickly to the laboratory where they will be analyzed. **Table 11** lists the nutrient concentration ranges considered adequate according to Brazilian researchers.

## 9. Green fertilization

Green fertilization is the cultivation of plants for the purpose of incorporating them into the soil. Among the desirable characteristics of a plant to be used as green manure, we may mention the possibility of mechanization from sowing to seed harvesting, absence of dormant seeds, vigorous and deep root system, ability to associate with nitrogen fixing bacteria in atmospheric air, fast growth to control weeds, and presence of mechanisms or synthesizing compounds that aid in the control of pests, such as nematodes, and diseases.

Several legumes have these characteristics, but generally there is a preference for *Crotalaria juncea* in the Center-South region of Brazil and for *Crotalaria spectabilis* in the states of Alagoas and Pernambuco, northeastern Brazil. *Crotalaria juncea* is a legume with a very fast initial growth, which provides it with a great competition potential with weeds. However, it is very sensitive to nictoperiods, early blooming in growing nights and, consequently, interrupting growth. Therefore, when cultivating for green manure, sowing should be performed in early October, or as soon as possible. However, for seed production, it should be sown in March.

In studies conducted by [1] in two regions of Minas Gerais, Alto Paranaíba and Zona da Mata, there was accumulation of dry matter (DM) by *Crotalaria juncea* sown in October, around 15 tons per hectare, with nitrogen concentration oscillating around 20 g of N per kg of DM. Thus, for a DM yield of 15 t $ha^{-1}$, the amount of N fixed and/or recycled is 300 kg per hectare. In areas densely infested with *Brachiaria plantaginea*, the inclusion of *Crotalaria* in the system increased the mass of N over the soil by 320% since the accumulation by the natural vegetation of the fallow area was 66 kg of N per ha, while in the area with *Crotalaria*, this accumulation exceeded 250 kg $ha^{-1}$, a sufficient quantity to ensure a production of 230 t of natural matter of sugarcane per hectare. Ref. [1] reported that in experiments conducted in areas where *Crotalaria* was incorporated into the soil, there was an increased productivity in plant cane of 15 t of culms per hectare compared to fallow areas.

The dry matter production of *Crotalaria juncea* and *spectabilis* in the states of Alagoas and Pernambuco oscillated around 4.5 t of DM per ha. This low production of DM, compared to that observed in the Center-South region, is mainly because the sowing season occurred at the beginning of the rainy season, between April and early May, therefore in longer nights. In Alagoas, in areas where *Crotalaria spectabilis* is used as green manure, it has been common to perform direct grooving without previous soil plowing, similar to the minimum cultivation systems adopted for some other crops.

## 10. Crop residues and sugarcane agribusiness waste

Straw is the main crop residue. There are also several types of waste from the industrialization of sugarcane, among them vinasse, filter cake, boiler ashes, and bagasse, which are routinely used in fertilization as sources of nutrients and organic matter. The amount of

straw that remains on the soil after the harvest of sugarcane not debrided with fire varies according to cultivar and adopted agricultural practices; such amount ranges from 12 to 18 t $ha^{-1}$ [22]. In studies conducted by [22] in the region of Ribeirão Preto, SP, it was verified that, among the nutrients in straw, only potassium presented a great liberation during 1 year of permanence of this crop residue in field (**Table 12**). Thus, with the exception of K, the nutrients contained in straw will not contribute significantly to the nutrition of sugarcane during the cycle following the cut.

Vinasse and filter cake are the main residues of cane industrialization. Vinasse, which has potassium, calcium, and organic matter as main constituents, is generally used for regrowth fertilizations and may, as discussed above, provide all the K for cultivation. According to the origin of the vinasse, the concentrations of the elements may vary, and chemical analyses must be conducted before its application. However, in general, the concentration of K in the vinasse originating from mixed must is, on average, twice as higher as that obtained from broth, with values ranging from 2.5 and 1.2 kg $m^{-3}$, respectively.

Filter cake has a high percentage of moisture (approximately 75%), and average levels of P and Ca vary, respectively, from 5.0 to 10 and from 15 to 36 kg per ton of dry matter. It is used mainly in plant cane fertilization, applied at the bottom of the planting groove at an average dose of 30 t of natural matter per ha, or in total area at twice the dose. Considering an application of 40 t of natural filter cake per ha, around 10 t dry matter, with an average content of 7.0 kg of P per t of dry matter, there is a contribution of 70 kg of P per ha, dispensing phosphate fertilization at the time of planting for most soils.

The composting of organic residues, mainly of sugarcane bagasse, is one more option for the use of such residues in the fertilization of sugarcane and in the improvement of the physical and chemical properties of the soil. The authors evaluated the technical and economic feasibility of using organic compounds based on sugarcane bagasse in sugarcane plantation. The research was conducted in soils with a great physical heterogeneity and a high capacity of phosphorus adsorption. Different mixtures of sugarcane bagasse and chicken litter were tested, ranging from 100 kg of bagasse to 80 kg of bagasse +20 kg of chicken litter, plus

| Year | DM (t $ha^{-1}$) | Nutrient (kg $ha^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg | S | C |
| 1996 | 13.9 a | 64 a | 6.6 a | 66 a | 25 a | 13 a | 9 a | 6.255 a |
| 1997 | 10.8 b | 53 a | 6.6 a | 10 b | 14 | 8 b | 8 a | 3.642 b |
| **Year** | **Structural carbohydrates (kg $ha^{-1}$)** | | | | | | | |
| | **Hemicellulose** | **Cellulose** | **Lignin** | **Cell content** | **C/N** | **C/S** | **C/P** | |
| 1996 | 3.747 a | 5.376 a | 1.043 a | 3.227 a | 97 a | 695 a | 947 a | |
| 1997 | 943 b | 5.619 a | 1.053 a | 2.961 b | 68 b | 455 b | 552 b | |

Source: Oliveira et al. [22]. Values followed by the same letter are not significantly different (Tukey's test) at the 0.05 level.

**Table 12.** Mass of dry matter (DM), amount of nutrients and structural carbohydrates in the samples of freshly harvested sugarcane straw without burning (1996) and in the remaining straw 1 year later (1997).

5.0 kg of ammonium sulfate. After the composting process, 15 t of material per hectare were applied to the bottom of sugarcane planting grooves. The fertilizer 06-30-24 was distributed over the compound at a dose of 500 kg per hectare. The results showed that the compound presenting the greatest productivity was the mixture of 100 kg of bagasse +5.0 kg of ammonium sulfate, resulting in an increase of 55 tons of culms per hectare compared to the treatment that received only chemical fertilization. The cost of production and the application of the compound were equivalent to 23.5 tons of culms, and the use of this compound allowed a net gain of 31.5 tons of culms per hectare. The results obtained in this study showed that even though sugarcane bagasse is a nutrient-poor residue, its effect on soil physical properties, especially aeration and water retention capacity, resulted in a higher productivity increase than that verified for compounds richer in nutrients. However, it also mineralized faster.

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Chapter 10

# Sugarcane: Organo-Mineral Fertilizers and Biostimulants

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Additional information is available at the end of the chapter

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**Abstract**

The combined application of organic fertilizer with mineral fertilizer increases the yield of sugarcane. It promotes greater residual beneficial effect in relation to the use of each fertilizer in isolation. The organo-mineral fertilizer presents gradual solubilization during the period of development of the crop. Thus, when compared to soluble mineral sources, its agronomic efficiency may be higher. Various types of organic material can be used, such as pig manure, poultry litter, filter cake and sewage sludge, among others. Organic matter is responsible for maintaining and increasing soil porosity to improve water retention and to ensure soil microbial balance. The efficiency in nourishing the sugarcane crops or availing the available nutrients is maximized. The use of biostimulants in world agriculture has achieved significant growth rates in the last decades. Hormone compounds ensure the sustainability of crops. It can be an alternative to improve plant nutrition, support of abiotic and biotic stresses. They act in the activation and potentiation of the metabolism of the cells, give more vigor to the immune system and help to enable the physiological processes in the different stages of development. The emergence and use of new technologies is the way to achieve greater productivity, sustainability and profitability.

**Keywords:** *Saccharum* spp., nutrition, nutrient cycling, plant hormones, sustainability

## 1. Introduction

Sugarcane (*Saccharum* spp.) is a species of plant in semi-perennial grass in the Poaceae family. It presents itself as an important culture providing not only food but also renewable energy. With all the existing technological package for the cultivation of sugarcane, it is possible to

envisage an even more promising future economically, socially and environmentally for the planet. Energy cogeneration, that is, the production of energy by employing more than one process, such as second-generation ethanol or cellulosic ethanol, is a great example. In this scenario, where the adoption of new agricultural practices and techniques has been done in an accelerated way, often passing the scientific production, it is necessary to produce knowledge that helps producers in the adoption of sustainable and economically viable technologies.

The quality and longevity of a cane field is related to soils of good chemical, physical and biological properties. A soil fertility management program is also a requirement for forming productive reeds. Thus, the addition and/or maintenance of organic matter in the soil as well as adequate levels of nutrients is necessary to obtain high productivities. Organo-mineral fertilizers contribute to the addition and maintenance of organic matter in the soil. Physiological factors associated with nutrition contribute considerably to the development of sugarcane plants [1].

In modern agriculture, additional techniques with the aim of obtaining the maximum productivity of sugarcane are being used. Among them is the use of biostimulants, regulators of plant or bioregulators. They act to activate the metabolism of cells, assist and confer greater vigor to the immune system, help to enable physiological processes at different stages of development, stimulate root growth due to the higher rate of cell development and induce the formation of new shoots, resulting in the potentialization of the quality and quantity of production [2].

Thus, this chapter aims to emphasize an adoption of sugarcane cultivation technologies such as the use of organo-mineral fertilizers and biostimulants. In this way, many studies have shown the great environmental, economic and sustainable benefits. The adoption of nutrient cycling from agricultural and urban waste can be an alternative and necessity in the present day.

## 2. Organo-mineral fertilizers in sugarcane

The great agricultural development in the twenty-first century increased the residues discarded. Thus, farmers began to reuse or cycling in larger proportions, realizing the great benefits of organic fertilizers and the advantages of mineral fertilizers have been combined. However, the economic feasibility of applying large volumes of organic fertilizers over large areas is still low. The addition of mineral components enriches the biofertilizer, providing high volume and cost reduction. Obstacles such as logistics, availability of raw material suitable for mineral source enrichment and production infrastructure are real problems that farmers and companies on the industry have to face. The knowledge of the composition and the handling of the residues constitute barriers that hinder the process.

The organo-mineral fertilizers are a mixture formed by fertilizers of organic and mineral fraction, characterized by a texture suitable for the supply to the crops. Existing information of specifications and minimum guarantees to know the best management and quantities to be used in each type of soil is necessary.

In Brazilian legislation, there are rules about its use and guarantees about its quality. It says that solid organo-mineral fertilizers should have a minimum of: 8% organic carbon; 80 $mmol_c\ kg^{-1}$;

10% isolated primary macronutrients (N, P, K) or a mixture (NK, NP, PK, NPK); 5% of secondary macronutrients; 1% micronutrients and 30% maximum moisture.

At European Union (EU), there are not maximum permitted levels of metallic elements on the composition of fertilizers. The European Commission (EC) is discussing the proposal to review the 2003/2003 regulation in order to impose limits on the content of minerals, heavy metals and organic fertilizers. The European regulation does not yet address environmental concerns arising from soil contamination from fertilizers. But, USA defends the imposition of limited values for As, Cd, Cr, Pb, Hg and Ni that are inserted on fertilizers [3].

Organo-mineral fertilizers significantly improve soil agronomic and environmental components for society. When it is applied in mulch fertilizers, they help to avoid high volatilization levels of ammonia ($NH_3$). In the other hand, mineral fertilizers have great solubility and are readily available to plants. The management of them should follow aimed criteria of their efficiency, economy and environmental sustainability.

The sources of the biofertilizers have great resistance to changes in its composition. This characteristic is transferred to soils that receive organic matter, allowing greater balance on the plant nutrition. The availability of organic carbon in the soil increases the microbial biomass, which improves the efficiency and release of nitrogen on the plants.

Organo-mineral fertilizers promote the union of the characteristics of chemical fertilizers with organic fertilizers [4]. Antille et al. [5], studying the effects of organo-mineral fertilizer derived from biosolids, granulated biosolids and urea, established that soils with ryegrass (Lolium perenne L.) that had the application of organo-mineral and organic biosolids had changes on the levels of P and increased its raise during 3 consecutive years. They also pointed out that there was a slow release of P with the application of this organo-mineral fertilizer. This release may work for several years from then.

The combined application of organic and mineral fertilizer improves the yield of sugarcane [4] and promotes greater residual benefits that will affect the relation on the use of each fertilizer singly. In this sense, the organo-mineral fertilizer presents reactive chemical potential relatively inferior to the mineral fertilizer. Its solubilization is gradual during the period of development of the culture, but when compared to soluble mineral sources, its agronomic efficiency may be higher.

After the homogenization, fractions of organic and mineral fertilizers can be extracted to a pallet capable of being supplied to the plants (**Figure 1**). Pelletizing is the process that fertilizer passes through a short period of time due to high pressure, humidity and temperature in order to reduce its size and turns it handling easier [6]. The production of the pellets (**Figure 2**) is objected to a high degree of hardness depending on the production process of the company, varying from 3.0 to 8.0 kgf $cm^{-2}$.

Carvalho et al. [7] state that organo-mineral fertilizers can improve the agronomic efficiency of fertilizers. These reduce the natural process of fixation of the labile phosphorus in the soil, being readily available to the plants for a longer time. Also, very mobile mineral components in the soil, such as nitrogen and boron, have its release slowed down by the organo-minerals, allowing its better leveraging by plants.

**Figure 1.** Organo-mineral fertilizer pallets. Source: Authors.

Some of the materials that can be used on the mixing or processing of organo-mineral fertilizer are sewage sludge, chicken litter or manure, bovine and porcine manure, castor bean cake, filter cake from the processing of ethanol and sugar, green manures, peat, organic compounds and fruit-processing residues.

de Sousa [9], doing a research about the use of organo-mineral fertilizer quotes in the production of sugarcane, concluded that there is higher efficiency on the use of nutrients applied through organo-mineral fertilizer than with mineral fertilizer. The same author also reports that organo-mineral fertilizer was more efficient in cane planted in the first year

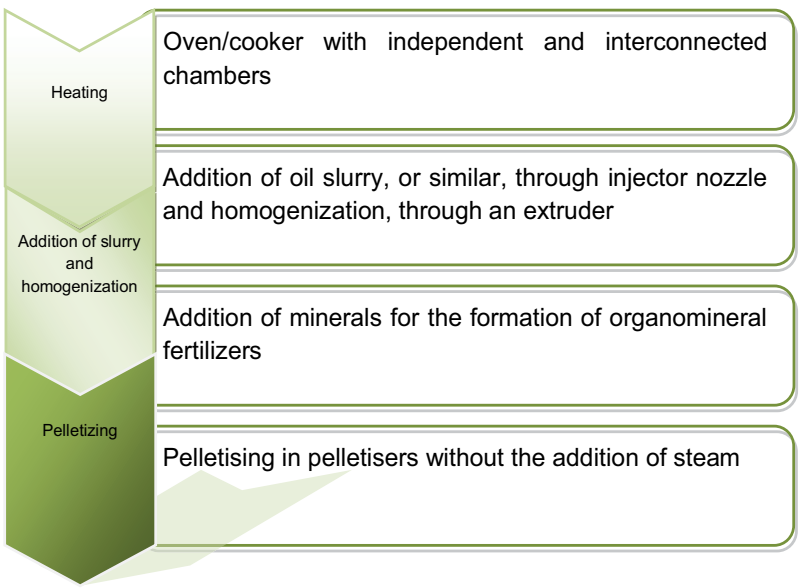


**Figure 2.** Flowchart of the physical-chemical-mechanical process of digestion and avian bed pelletization for the production of organic compound and organo-mineral fertilizers. Source: de Oliveira [8].

than in cane budded after the first year. This fertilizer can substitute the mineral fertilizer, because it can increase efficiency up to 24% of stem production. In another study, Teixeira et al. [10] also observed great efficiency in phosphorus doses provided by organo-mineral fertilizer in the sugarcane crop. de Sousa [9] comments about some "sustainable benefits of organo-mineral fertilizers," and some of these benefits are recovering capability of the microbial flora, the reduction of soil acidification and the gradual release of nutrients. Such benefits will influence the best development of root system, lower fixation of phosphorus to soil colloids and better water retention. It also worth nothing to the operational cost of crop. This use reduce operations for fertilization with mineral and organic fertilizer together throughout the crops.

Gurgel et al. [11] studied BIOFOM (an organo-mineral biofertilizer formulated with concentrated vinasse, filter cake, boiler ash and chimney soot and complemented with mineral fertilizers), a technology for the reuse of trash from the sugarcane agroindustry and pelletized organo-mineral fertilizer, and they concluded that organo-mineral can replace the partial or total fertilization of sugarcane crop. Another major benefit is the reduction of the production and accumulation of industry trash.

## 3. Biostimulants in sugarcane

The use of biostimulants in world agriculture has achieved significant growth rates in the recent decades. There are estimations that by 2018 this market will move US$ 2.2 billion. It means a growth rate of 12.5% from 2013 to 2018 [12]. There are products that promote sustainability of crops and can be an alternative to improve plant nutrition, support of abiotic and biotic stresses and is efficient in integrating pest and disease control. Some biostimulants have indirect pest control properties that do not fit in the insecticide regulation. Others have indirect fertilizer properties containing micronutrients that are better to foliar fertilization.

Most of the biostimulants contain synthetic plant hormones and fertilizers. Vegetable hormone is a natural compound produced in the plant with organic characteristics. Synthetic vegetable hormones, also called plant regulators or bioregulators, are artificially produced compounds with organic characteristics and can be supplied to the vegetable.

Vegetable stimulants or biostimulants are mixtures formed between plant regulators and other biochemical compounds, such as amino acids, nutrients and other active ingredients, which can contribute to plant development [13].

It is known that plants are influenced by internal and external factors. We can cite the external factors such as light, temperature, rainfall, photoperiod, soil type, fertility and so on. Internal factors of a chemical nature regulate plant growth. The mechanism of regulating and/or controlling the development of animals and plants depends on information passed between cells, tissues and organs. These metabolisms control substances that emit chemical signals that are called hormones [13, 14].

The plant hormones can be produced in a tissue and transported to another part of the vegetable where its action will take place. Some of these hormones are called phytohormones,

and they are produced in the vegetable in tiny quantities and very small proportions. The same hormone can trigger different responses or reactions in different organs and stages of development of the plant. In plant hormones, there are interactions since they hardly act isolated. The auxins, cytokinins, ethylene, abscisic acid and gibberellins (GAs) are traditionally the five most well-known phytohormones. Brassinosteroids, salicylic acid, jasmonic acid and sistemin are other substances that also emit recently researched chemical signals [14].

The biostimulants are the mixture of hormones with different plant regulators or with nutrients that can provide better performance for plants. The presence of plant hormones promotes vital and structural changes in the plant. Thus, there will be better cellular development and tissue growths. On an objective way, organs such as leaves, stems and roots can develop in larger size and number reflecting on the plant's production potential. On a positive way, nutrients combined in/or association will have better effects. The biggest potential production joining with the available nutrients can promote greater effect on the productivity of crops such as sugarcane.

In a study of the productivity and technological quality of sugarcane ratoon with the objective of application in the plant growth regulator and liquid fertilizers, Silva et al. [15] observed that genotypes respond differently to the use of biostimulants in the absence or presence of foliar fertilizers in sugarcane after the first year of harvest.

The application of 0.09 g $dm^{-3}$ of kinetin, 0.05 g $dm^{-3}$ of 4-indole-3-ylbutyric acid and 0.05 g $dm^{-3}$of gibberellic acid and liquid fertilizers has no effect on the technological quality of sugarcane juice. The use of the hormonal mixture in the absence and presence of liquid fertilizer increases the yield of sugarcane and the amount of sugarcane. Raposo et al. [16], in evaluation of different foliar fertilizers on the crop production of sugarcane associated with biostimulants, concluded that the association of micronutrients plus biostimulants is increased by 17% in sugar yield.

### 3.1. Auxin

Charles Darwin and his son Francis, in their book published in 1881, mentioned studies involving growth regulators. Some years later, in 1926, Frits W. Went designated the substance that involved his studies of auxin, being the first hormone described in the literature [14]. Meristematic tissues of plants are the main production sites, either in the airways or underground. Depending on the tissues or production sites, there are large variations in the quantities produced [13].

Auxin indole-3-acetic acid (IAA) is one of the major plant hormones (**Figure 3**) produced in the plant that has great capacity to influence on its growth and initiation of exchange activity and apical dominance [17]. The IAA regulates cell division and expansion, vascular differentiation, lateral root development and apical dominance [18]. Indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA) and phenylacetic acid (PAA) may also be referred to as the auxins of plants [19]. Lisboa et al. [20] verified a viable result for the development of sugarcane corns using 0.125 mg/l of 2,4-D and concluded that auxin decreases the diameter of the cell and its nucleus.

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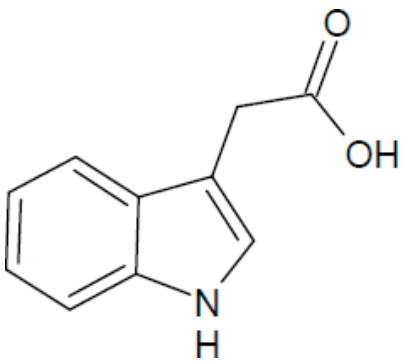


**Figure 3.** Indole-3-acetic acid (AIA) which is the main naturally occurring auxin. Source: Raven [14].

### 3.2. Kinetin

Cytokinins (**Figure 4**) began to be discovered by Johannes van Overbeek around 1941 when he observed that coconut water (Cocos nucifera) promoted embryonic development and growth of cells and tissues. The natural cytokinins are 6-*N*-substituted purine [21], and their isolated use has little or no effect. Its action is closely related to auxins and acts as a stimulant of cell division. With the union of the two hormones, there is a fast division of cells forming a large number of small and undifferentiated cells. But everything will depend on the concentrations and proportions of both hormones. At high concentrations of auxins, there will be a great root formation. When in high concentrations of kinetin, there will be gem growth. In equal concentrations, there will be production of meristematic cells. Cytokinins are also involved in the establishment of functional root nodules, which influence the nutritional status of the plant and may interfere with flowering time.

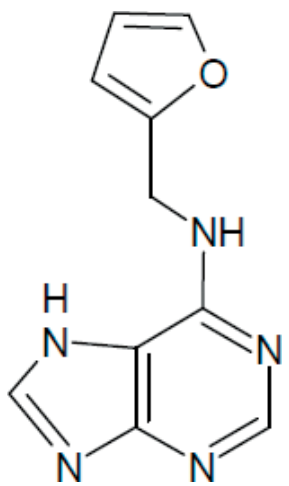


**Figure 4.** Molecule of kinetin that probably does not occur naturally in plants. Source: Raven [14].

Controlling the rate of differentiation and cell division, the cytokinin determines the size of the meristem root. Thus, there is a balance of auxin effects that is responsible for controlling cell division [14]. The cytokinins still delay the aging of the leaves, avoid their senescence and prolong their useful stage in the plant. Raposo et al. [22] comparing media productivity of sugarcane noticed that the addition of kinetin to coconut water promotes cell regeneration and growth of sugarcane.

### 3.3. Gibberellin

Gibberellins (GAs) were discovered by Japanese scientists in 1926 (**Figure 5**). These substances are present in practically all plants being found in 136 natural gibberellins. GAs are a class of phytohormones that regulate various sites and stages of plant development. The main actions in the plant such as stem elongation, germination, flowering and fruit development can be mentioned [23].

Gibberella acid is the most studied gibberellin produced by the fungus *Gibberella fujikuroi*. It promotes cell division and stretching and causes noticeable stretching of stems, roots, leaves and fruits. They are efficient to overcome dormancy and promote seed germination [14]. Alcantara et al. [24] studied about shoot multiplication, elongation and rooting in vitro of clones of sugarcane under different concentrations of 6-benzylaminopurine and gibberellic acid and concluded that the gibberellic acid in the elongation of the seedlings varies according to the genotype; for the clones RB036152 and RB036066, it promotes the formation of larger seedlings.

### 3.4. Humic acids (HA)

The decomposition of animals or plants is a part of an organic cycle. Humic substances (HS) are produced when it happens. Some researchers argue that HS improves the biological, chemical and physical quality of soil and the physiological development of plants. There is a difficulty in understanding the HS action on plants due to the complexity of the chemical mixture. The improvement in nutrient absorption efficiency has been the most widely held idea. They may increase permeability of the cell membrane favoring the ionic transport in the cell. There is also a higher efficiency in the products generated from the Krebs cycle (ATP) as a result of the increasing of respiration and the speed of enzymatic reactions. This

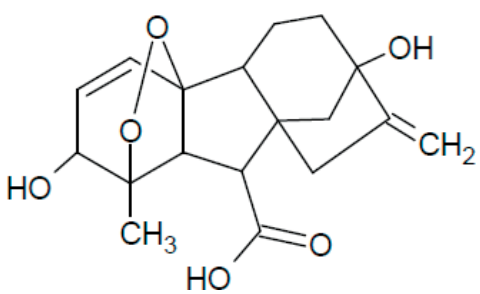


**Figure 5.** Molecule of gibberellic acid that is more abundant in fungi and the most biologically active in many tests. Source: Raven [14].

directly influences the development of the plant. Morozesk et al. [25] explained that the increase on the efficiency of nutrient absorption is related to the activation of $H^+$ − ATPase (proton pump activity). Thus, products such as humic acid (HA) can interfere positively on physiological phases and guarantee better efficiency in plant nutrition, especially in the early stages [26].

Marques [27], using humic acids and diazotrophic endophytic bacteria in the production of sugarcane, obtained increases of up to 23% in the productivity of foliar application in sugarcane variety RB867515. Civiero et al. [28], in the study application of humic substance and L-glutamic amino acid in different sizes of 1-bud set of sugarcane, noticed superiority of the humic substances to L-glutamic acid and control for the variable root length, root surface area, dry mass of root system and dry mass of aerial part. Leite [29] concluded that in general, urea + HS doses promoted a significant increase of 6% yield of sugarcane stalks and a 4.5% increase in sugar production (Mg $ha^{-1}$), comparing only to the application of urea doses.

### 3.5. Fulvic acids (FA)

There is a division of different categories of humic substances between humic acids, already cited and fulvic acids (FAs). There are commercial types of biostimulants divided between two types of acids. In general, [12] cite some authors that discuss about the size of the molecules. FAs are considered larger molecules with higher molecular weight. The fulvic acids are the organic fraction of the soil soluble in acid and basic solutions. Also, they have higher acidity and carboxylic groups, conferring an important characteristic such as the better capacity in the exchange of cations. Other important characteristics are the abilities of chelation and mobilization of metallic ions, mainly Fe and Al. Yet, AFs do not have selectivity of plasma membrane different from humic acids.

Exposed this large amount of characteristics, the authors [12] reported that in corn plants (Zea mays) there is greater root development, reduced transpiration and Al toxicity, in soil with high Al concentrations, increase in the production of biomass and nutrient absorption and better performance under Water stress. In wheat crop (*Triticum aestivum*), higher growth and plant weight, improved nutrient uptake, higher amount of chlorophyl, reduction of water stress, and increases the absorption of phosphorus can be observed. In rice (*Oryza sativa*) can be noticed a greater efficiency in the absorption of iron. Common bean (Phaseolus vulgaris) improved the development of adventitious roots and reduction of lead toxicity in the exposure of high loads of this. There were no reports of specific effects of fulvic acids in sugarcane.

### 3.6. Silicon as biostimulant

Silicon as a biostimulant has a good availability in soils in the form of oxides of silicon. Then, it can be concluded that the lack of silicon is not limited to the cultures. However, the majority of the sources present in the Earth's crust are poorly soluble and insoluble in water. So it is difficult to find readily soluble sources that are economically viable. In the other hand, there are industrial wastes on the extraction of silicates, which have been studied and shown efficient use

as fertilizer and/or stimulator. This requires special care, mainly because of the risk of contamination with heavy metals, fact unwanted for the crops. The use of these residues should occur only with the removal of these contaminants or with the purification of the silicon source. The other problem is the very low mobility in the phloem of plants.

Some plants, like the dicotyledons, do not accumulate this nutrient on the tissues. But, in the other hand, sugar cane and other grasses have ease of absorption and accumulation of Si (**Figure 6**). The effects of biostimulants are a result from deposition within the tissues, specifically in the cell wall which increases the thickness, stiffness and lignification of cells. This confers better resistance to biotic and abiotic stresses. They are also physical benefits to the barriers of silica on the fabrics, giving best architecture to plants with leaves more upright and reducing shading which improves photosynthetic efficiency, since there is a reduction in the rates of transpiration.

Lower transpiration implies less demand of water by plants and reduction in levels of damage caused by fungi and insects phytophagous Lepidoptera. In another analysis [31], the plants are submitted to several physiological and metabolic diseases. There are many other discoveries that show interference in the activity of some enzymes, reducing the antioxidant capacity of some oxidative compounds, interfering in relations of water in the plant, photosynthesis, absorption of nutrients, mobility of ions inside of the plant tissues, hormone balance and in gene expression. It reports that Si increases the concentration of some metabolites nonenzymatic acting and defensively against oxidizing agents.

The use of fertilization facilitates the action of the plant to regulate the nutritional balance. Besides the ability to regulate the absorption of Zn in the presence of high levels of P, can be prevented the onset of symptoms of deficiency of Mn and B; reduces the absorption of Na in plants exposed to high concentrations; reduces the toxicity of heavy metals and Al forming a link themselves metal. From the physiological point of view, there is a great efficiency of Si in avoiding or reducing the permeability and selectivity of the plasmatic membrane at the input and output of ions under

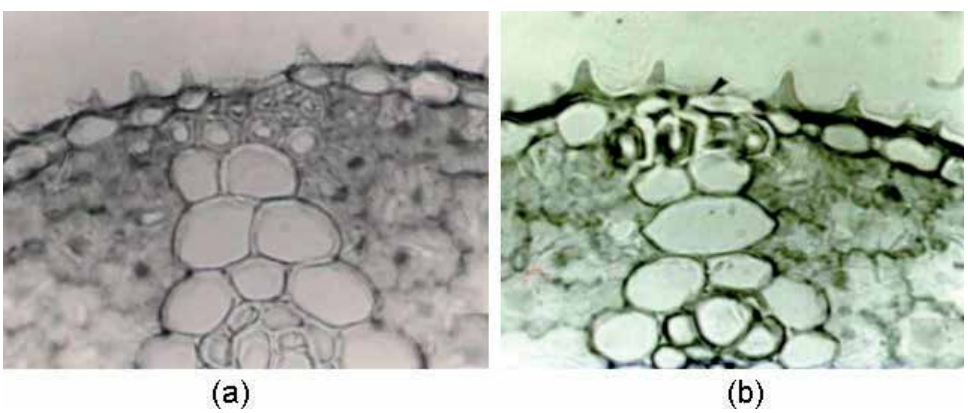


**Figure 6.** Transverse cuttings of leaf blade limb of rice plants (Oryza sativa L.). (A) Detail showing projection of the external wall of the epidermis on the adaxial side (×1000) of the leaf limb treated with 5 mg of N and zero of $SiO_2$ (×400). (B) Detail showing projection of the external wall of the epidermis on the adaxial side (×1000) of the leaf limb treated with 5 mg of N and 400 mg of $SiO_2$ (×400). The tip of the arrow at B indicates spherical silica bodies. Source: Mauad et al. [30].

conditions of stress. The supply of Si enhances the extension of the cell wall, and root system may cause an increase in the rate of absorption of nutrients. Other studies have shown that the presence of other biostimulants influences on the final amount of plant hormones. In soybean plants, stressed with higher salt concentration, the levels of gibberellin increased with the objective of supplying themselves needs. In another study cited by Savvas and Ntatsi [31], the Si has reduced the levels of jasmonic acid and salicylic acid in rice plants exposed to the stress of heavy metals. The abscisic acid can also cause negative or positive effects in the presence of each other. These phytohormones play an important role in the regulation of physiological processes and in the control of biotic and abiotic influences. Lately, it has cause a great improvement in the understanding of the features of Si in the interior of the plants. However, there seems to be a need to improve these skills.

## 4. Conclusion

The proper nutrition of sugarcane is very important to obtain high production of the crop. With the use of organo-mineral biostimulants and fertilizers in association, the global importance of the improvement of this technology stands out. The productivity gains in sugarcane plantations are notorious. The big gains come from building and maintaining a fertile soil to the cycling of essential nutrients that are discarded as trash. There is, also, a reduction of the contamination of fountains and subsoil, reduction of the emission of gases that cause greenhouse effect, and reduction of proliferating environments of diseases and their respective vectors.

The environmental, economic and social gains are great when this agricultural practice in the cultivation of sugarcane is used. The emergence and use of new technologies are ways to achieve greater productivity, sustainability and profitability. Several technologies on the use of plant hormones, especially synthetic ones, have contributed to these goals.

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Chapter 11

# Mathematical Optimization Models in the Sugarcane Harvesting Process

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Carlos Alexandre Costa Crusciol and
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Additional information is available at the end of the chapter

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**Abstract**

Over the past few decades, due to environmental and economic factors, the sugarcane has been considered a versatile and important plant to the several countries. The energy-sugar-ethanol agro-industries are seeking to take advantage of all its material, with the main products produced being renewable energy, sugar and ethanol. In this chapter, we propose to present a review of the important works that use mathematical and computational tools, aiming to optimize the sugarcane harvesting, in the past 30 years.

**Keywords:** economy, mathematical models, optimization, sugarcane, mechanized harvesting

## 1. Introduction

A number of environmental and economic benefits are claimed for sugarcane. Currently, the ethanol is the most widely used biofuel for transportation worldwide. Production of ethanol from sugarcane is one way to reduce consumptions of both crude oil and environmental pollution. In addition to ethanol, sugar and renewable energy can also be produced from sugarcane. In this way, sugarcane is considered one of the most important industrial cash crops of the world.

On the other hand, there is a great concern about some factors related to the sugarcane production, for example, to increase the processing capacity of the large volume of sugarcane; control the pollution; improve the sugar content and quality of the harvested cane crop; reduce the losses and to increase the volume of the load in the transshipments and trucks. In this context, much research has been carried out in an attempt to improve the

production process in this sector, especially with regard to the sugarcane harvesting [1]. Many studies have been performed in the sugar-energy sector using mathematical and computational modeling techniques in the mechanized harvesting planning. These studies present methodologies to optimize the sugarcane harvesting planning aiming to maximize sugarcane production; minimize costs related to harvesting; minimize the number of maneuvers of the harvester machine; optimize routes for the transport of machines and trucks and many others. The mathematical tools use continuous, discrete and heuristic optimization techniques [2–7].

According to Sethanan and Neungmatcha [2], one of the important aspects to increasing sugarcane mechanized harvesting efficiency is the optimal planning of the harvesting. These authors noted that minimizing the distance traveled during the harvesting and maximizing the sugarcane production, many economic and environmental gains are achieved. However, these are difficult task to implement because there are conflicting objectives that need to be considered simultaneously. Most of these and many other aspects of the sugarcane industry make their management very complex. In addition to the intrinsic knowledge on the part of the managers, the agro-energy industries have sought partnerships with researchers from universities and research centers to assist them in the development of an optimized crop management. In this way, the development of scientific methodologies such as mathematical modeling and operational research (OR) techniques to aid in decision-making has been very important in this area [7, 9].

Based on the above discussions, we propose in this chapter to present a review of the important works that use mathematical and computational tools, aiming to optimize the management of sugarcane, in the past 30 years.

This chapter is structured as follows. Section 2 describes the evolution of mechanized harvesting in the world since the 1940s. Section 3 presents the world scenario for sugarcane production and harvesting. Section 4 describes the types of harvesting in several countries, as well as their advantages and disadvantages. Section 5 presents the relevance of the mathematical optimization models applied to the sugarcane harvesting process.

## 2. Evolution of the mechanized harvesting of sugarcane

There are reports on the use of mechanization in sugarcane harvesting since the 1940s; however, due to the great loss of raw material caused by the first harvester machines, mechanization did not gain importance in this period, predominating manual harvesting until the 1950s [10–12].

From the 1960s to the 1980s, there was a great increase in the use of mechanization in the sugarcane harvesting. In the 1960s, some countries, such as Australia, used the mechanized system in about 80% of the sugarcane harvesting [10, 11]. At the end of the 1970s, the sugarcane harvesting in Australia reached 100% of mechanization [13]. In some other countries, the mechanized system was introduced only in the late 1980s, due to labor shortages, economic and environmental problems [14]. Mechanization requires large initial capital

investments, however, increases production and significantly diminishes labor requirements and costs.

The fuel crisis (the search for alternative fuel sources, for example, ethanol) and environmental (reduction of burning in sugarcane plantations), social (labor issues) and economic issues led other countries to join the mechanized harvesting system from the 1990s. In this way, the mechanized harvesting was introduced in the scenario of the sugarcane industry. After that, many and great improvements have been observed, such as the increasing volume of the sugarcane harvested, the industry became able to meet the demands, studies have been made aiming the performance improvement of the machinery and equipment, and the pollution generated by the pre-harvest burning of sugarcane has been reduced. Therefore, the mechanized harvesting grew in synchrony with the technological evolution, forced by the demand of the consumer market and the environmental impositions [15–17].

## 3. Sugarcane in the world

Sugarcane is a semi-perennial crop and is produced in several regions in the world. According to Kim and Dale [18], in the past, the main uses of sugarcane in the world were basically for food manufacturing and seed extraction. Over the years, the sugarcane started to be looked as an energy feedstock rather than a food and this fact made its production grow significantly. The global evolution of the area planted with sugarcane and the amount harvested for mechanized and manual harvesting are presented in **Figure 1**.

Brazil has remained the world's largest producer of sugarcane since 1970, followed by India, China and other countries (**Figure 2**).

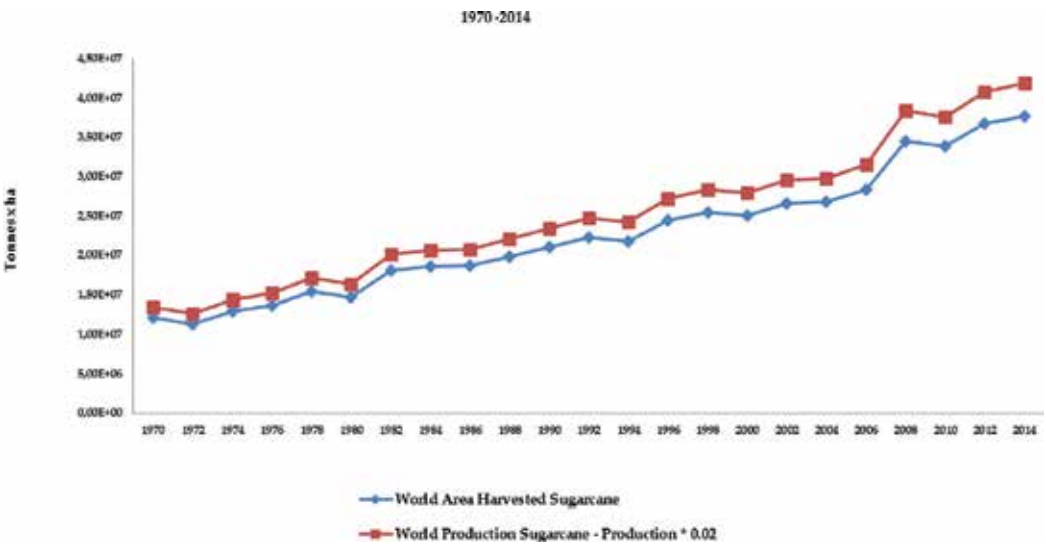


**Figure 1.** Sugarcane production in the world [19].

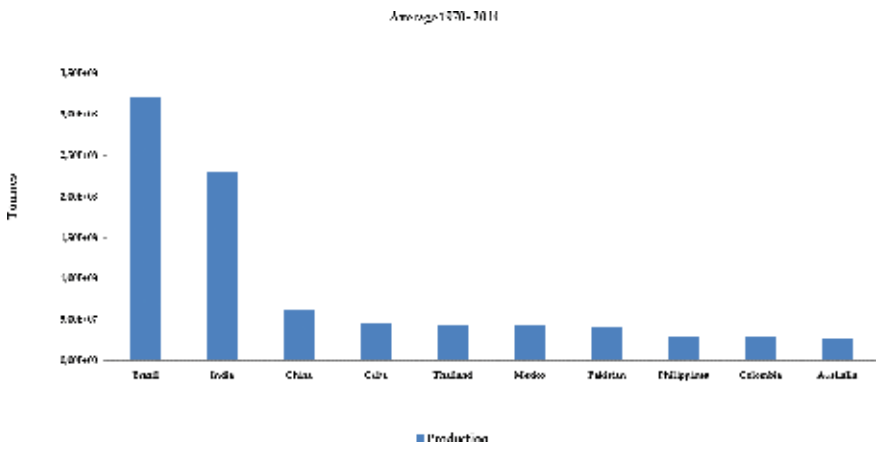


**Figure 2.** Top 10 producers of sugarcane [19].

Therefore, it is evident the importance of sugarcane for the economy and sustainability of several countries in the world.

## 4. Sugarcane harvesting

Sugarcane cultivation has been strengthened in some regions of the world, such as North America, Central America, South America, Asia and Oceania, due to the climate, temperature, humidity, relief, topography and soil type. In these countries, planting and harvesting of sugarcane were first carried out in a rudimentary way, manually, as shown in **Figure 3**.

Even with the evolution of sugarcane harvesting technology, there is still manual harvesting practice. In countries, such as United States (Louisiana, Hawaii, Texas and Florida) and Australia (Queensland), the sugarcane has been mechanically harvested since the mechanization of the sugarcane became feasible; however, in others countries, such as Brazil, Argentina, Colombia, Indonesia, among others, the mechanized harvesting was slowly developed and the manual harvesting is present in part of the cane fields until now. In these countries, the transition from manual to mechanized harvesting has been required to improve productivity and to meet labor and environmental issues [20–22].

The sugarcane harvesting can be done with the raw cane or burned cane. In general, a preburning of the straw is performed prior to manual cutting of the sugarcane. This practice is used to clean the cane, making it easier and safer for manual laborers to work. Some countries also use mechanized harvesting with the burned cane. The burning of the sugarcane is a common practice; however, it is very widely criticized due to environmental and productive factors. Therefore, mechanized harvesting of raw cane (**Figure 4**) is more commonly used nowadays, and is a focus of research worldwide. Researchers search for a new approach to the sugarcane mechanized harvesting that could make it more economically and environmentally attractive [23–26, 27].

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**Figure 3.** Hand sugarcane harvesting. Credit: Luiz Carlos Dalben.

**Figure 4.** Sugarcane mechanized harvesting. Credit: Luiz Carlos Dalben.

The authors [14, 28] describe the operation of the sugarcane harvester, which can be categorized into whole stalk harvesters and chopper harvesters. The sugarcane harvester machines perform the basal cutting, promote the cleaning of sugarcane and chop the stalks into 15–40 cm billets, unloading them onto a transshipment (**Figure 5**). Additionally, the sugarcane is delivered to a train or a truck and transported to the processing center.

The mechanized harvesting of the sugarcane is carried out annually and each machine cuts approximately 80 tons per hour. Depending on the number of hours worked, it can cut annually between 50,000 and 150,000 tons per harvester [20].

Thailand is the second largest exporter and the fifth largest sugarcane producer in the world. However, most sugarcane farming is family business, hence sugarcane is cultivated in a small area, which makes mechanized harvesting unfeasible and promotes low productivity [28, 29]. According to Pongpat et al. [23], despite the great importance of sugarcane to Thailand's economy, the population has been aging and it has been difficult to meet the significant market demand using only manual harvesting. It is necessary to review the concepts and apply new investments in the mechanization of harvesting in this country.

In Cuba, sugarcane is considered the second largest source of economy, has hundreds of mills and produces millions of tons of sugar per year; however for this, the integrated harvesting, transshipment and loading system work efficiently [30].

Sugarcane has a great economic importance in Australia. According to Higgins and Davies [31], in this country, the sugarcane is mostly concentrated in the northeast, and the cut begins in the winter and goes until the end of spring, when the highest percentage of sucrose is concentrated.

**Figure 5.** Transshipment to aid the transport of sugarcane from the plot to the truck or train. Credit: Luiz Carlos Dalben.

According to Braunack et al. [33], the traffic of machinery in the sugarcane plantation is very intense and requires a good planning of the harvesting process to avoid problems of harvest delay, loss of sucrose, soil compaction, delayed delivery of harvested sugarcane and many others. In [34], the quality of sugarcane harvested manually and mechanically is compared. They conclude that in both cases that after the cut, the sugarcane must be quickly taken for processing because after 24 hours the loss of quality begins. The logistic integration of harvesting, transshipment and transportation must be in constant harmony, aiming to optimize the time between cut and milling in the mill, i.e., there must be an efficient communication network and a good harvesting planning. Therefore, researchers in various parts of the world investigate effective and economical ways to manage the process of harvesting sugarcane. Many of these researchers make use of mathematical and computational methodologies to optimize this process.

## 5. Optimization process

Investments in technology have grown considerably in developed and developing countries, mainly investments in technologies aimed at agricultural machinery, including sugarcane harvesting machine. Due to these investments, the machines have become more agile and productive, promoting a considerable increase harvesting yields, and consequently forcing managers to make faster decisions during the process of mill management. Therefore, many studies were directed towards the development of optimization mathematical models as a way to assist managers in decision-making.

### 5.1. Mathematical models

Since the 1970s, many mathematical models have been developed aiming to optimize the mechanical harvesting process of sugarcane

In 1977, Gentil and Ripoli [35] analyzed and simulated the mechanized harvesting system, transport and additionally, the reception of sugarcane in the mills. The logistics of transportation and harvesting of the sugarcane were optimized aiming to reduce the time involved in the harvesting process and the number of vehicles (harvesters and trucks). Despite the computational limitations, promising results were obtained, considering the dimensions of the problems of this time.

In 1982, Singh and Abeygoonawardana [36] developed an optimization model for the harvesting and transport of sugarcane, aiming to optimize the number of trucks for the transportation of harvested sugarcane in mills in Thailand.

In 1994, Singh and Pathak [37] presented an optimization model-based decision support system and simulation of the harvesting operation, aiming to minimize harvesting costs and aid the optimal management decision-making for the mechanized harvesting of sugarcane.

In 1995, Semenzato [38] used a heuristic to simulate the sugarcane harvesting, aiming to assist the decision maker to optimize cutting, loading, transport and discharge time. The results achieved helped in making optimized decisions aiming at the organization and use of scarce resources.

In 1999, Askita et al. [39] developed a scheduling algorithm called SFSW (Stochastic Farm Work Scheduling Algorithm based on Short Range Weather Variation) to assist Japan's sugarcane industry in determining the optimal daily amount of sugarcane to be harvested and deciding which fields to perform the operation of harvest. This algorithm was considered quite promising when was compared to real practices.

In 2000, Díaz and Perez [30] considered that to optimize the harvesting and transportation of sugarcane, involving the cutting and loading of the truck is a complex task. Therefore, these authors proposed a computational simulation aimed at the optimization of sugarcane harvesting and transportation. The results found contributed to the development of optimal planning of sugarcane processes.

In 2001, Arjona et al. [40] observed some problems in Mexican sugar-energy sector related to the underutilized machines and difficulties presented by farmers to plan the sugarcane harvesting. These authors developed a computational simulation of the harvesting, transportation and sugarcane processing systems, aiming to aid managers to plan and evaluate actions with a computational tool. The results of this research allowed the correction of the problems underutilization of machinery and the minimization of costs, fuels and processing time of sugarcane.

In 2002, Higgins [41] proposed an integer linear programming model to optimize the number of harvesters to be used at five Australian mills. The author describes the great importance and benefits that mathematical modeling can promote to power mills. Higgins and Muchow [42], in 2003, also explored operational research techniques to increase productivity and profit in sugarcane production and harvesting.

In 2005, Higgins and Davies [43] emphasized the complexity of mechanized harvesting and transportation in the sugar-energy sector. They proposed a stochastic model to evaluate scenarios of cost reduction in mechanized harvesting and transportation. The results allowed to obtain a more efficient transportation service and with greater benefit to the harvest. Jiao et al. [44] proposed a linear programming model to improve crop planning in order to optimize the amount of cane to be cut per farm and the sugar content. As a result, a software called SugarMax was introduced with the purpose of assisting in decision-making.

In 2006, Higgins [4] proposed a mixed integer linear programming model with the objective of reducing the queuing time of the trucks and optimizing the harvesting process. The computational tests were performed using the GAMS software, OSL and heuristic techniques. Milan et al. [45] studied the transport of sugarcane, involving numerous variables and constraints, such as decisions of the continuous milling, harvesting machining, number of vehicles used to transport sugarcane and available routes. The model was designed to minimize transport cost and harvest limitation.

In 2007, Grunow et al. [32] investigated the safety stock of sugarcane to be used as raw material for sugar production. The problems of cultivating farms, harvesting, dispatching and harvesting equipment were analyzed. A mixed integer linear programming (MILP) model was proposed for the mechanized harvest planning, optimizing the weekly milling of sugarcane and the amount of sucrose and allowing a more detailed harvest schedule with small sucrose losses.

In 2008, Salassi and Barker [46] developed a study aiming to reduce costs and minimize harvesting time. In this way, a mathematical programming model was developed, which provided the ideal harvest time under different waiting times.

In 2009, Jena and Aragão [47] proposed an integer linear programming model to optimize harvesting. In order to facilitate the resolution of the problem, heuristic initial solutions were obtained and exact methods were applied with the use of CPLEX and other software, obtaining an improvement of almost 25% in the total average of cane production. The authors recommended the use of mathematical techniques for this type of problem.

In 2010, Scarpari and Beauclair [9] also used linear programming and the General Algebraic Modeling System (GAMS) software to maximize profit and harvesting time for sugarcane.

In 2012, Stray et al. [48] formulated a model of optimization based on traveling salesman problem aiming to determine an optimal planning of the sugarcane harvesting involving large number of fields and extensive areas of planting. The researchers concluded that the decision support system provides practical support for sugarcane harvesting; however, even then, numerous researches are needed in this area.

In 2013, Silva et al. [49] developed and applied a Multi-Choice Mixed Integer Goal Programming Model (MCMIGP) for a real problem of production planning in a sugarcane mill, extending to mechanized harvesting. The authors argue that mathematical techniques are good tools to assist power plant managers in making decisions. Sethanan et al. [50] presented an optimization model applied to sugarcane harvesting aiming to maximize sugar production in the harvest period. The authors presented a heuristic to schedule the sugarcane harvesting and a Tabu Search algorithm to optimize production. The results showed an improvement average of 16.38% in sugar production. Jena and Poggi [8] presented an optimization model for tactical and operational planning such that the total sugar content in the harvested sugarcane is maximized. The model was solved using heuristic techniques and approached Lagrangian relaxation or Benders decomposition.

In 2014, Florentino and Pato [5] presented a bi-objective binary linear programming model for sugarcane variety selection and harvesting residual biomass utilization. The computational experiment showed a high quality of the proposed multiobjective Genetic Algorithm and a low computational time. The authors concluded that the mathematical techniques could aid the managers of mills in the strategic planning process of productive activities of the sugarcane. Silva and Marins [51] proposed a Fuzzy Goal Programming (FGP) model to optimize storage and transport logistics of sugarcane involving uncertainties in the agricultural process of sugar and ethanol production. The results indicated that the presented methodology could assist the managers in the decision making, mainly to the processes related to the harvesting, transshipment and transportation of the sugarcane.

In 2015, Silva et al. [52] proposed a Revised Multi-Choice Goal Programming (RMCGP-LHS) model to address uncertainty in sugarcane harvesting planning, production planning and energy cogeneration for a sugarcane mill. The model addresses the agricultural and industrial stages, allowing the decisions to be taken within a weekly planning horizon, including the process of variety selecting of the sugarcane to be planted, the design of the cutting front and the agricultural logistics, as well as the choice of the production process of sugar and ethanol.

The objectives of this model are to obtain information to harvest the sugarcane in the period closest to the maximum sucrose content; minimize agro-industrial costs and maximize the production of sugar and ethanol and the sale of energy. Neungmatcha and Sethanan [53] carried out studies on optimum planning of the mechanized harvesting route in order to improve transportation. These authors proposed a mixed integer model aiming to increase profits and reduce costs through the better supply of sugarcane and more efficient mechanized harvesting and transportation. Kittilertpaisan and Pathumnakul [54] studied problems related to the mechanized harvesting of sugarcane in Thailand. A mathematical model related to the problem of routing of vehicle was formulated. Harvest sequences, routes, harvesting period and harvesting time were successfully determined.

In 2016, Ramos et al. [3] proposed a methodology to determine an optimum planning for planting and harvesting of the sugarcane for 5 years. The main decisions approached in this methodology are related to the determination of the planting date, selection of the varieties to be planted and determination of the harvest date for each plot, aiming to optimize the global production. A binary nonlinear optimization model was proposed and solved using computational and mathematical strategies, ensuring that the date of harvest is always in the maximum maturation period of sugarcane and considering all operational constraints of the mill. An optimal planning was determined, obtaining a potential improvement production of sugarcane 17.8% above the production obtained by conventional means.

In 2017, Junqueira and Morabito [55] proposed an optimization approach to support decisions from the scheduling and sequencing of harvesting fronts using the General Lot Sizing and Scheduling Problem (GLSPPL). Santoro et al. [56] proposed a mathematical model to solve the route planning problem of the sugarcane harvester, which aimed to optimize the time of maneuver of the harvesters in comparison to the maneuvers that were being commonly used. Based on the presented results, a 32% time reduction was observed compared with the traditional harvest process for the same area when the route of the harvest machine was not planned. Florentino et al. [57] proposed a methodology to aid the planning of the sugarcane harvesting aiming to improve the sucrose production and the raw material quality, considering the constraints imposed by the mill as well as the sugarcane demand per period. In this way, an extended goal programming model was proposed to optimize sugarcane harvest planning, so that the harvesting is done as close as possible to the sugarcane maturity peak. A genetic algorithm (GA) was developed in order to solve large-size problems with an appropriate computational time. A comparative analysis between GA and an exact method for small instances was given to validate the performance of the model and the methods developed. The computational results show that crop planning for small farms can be generated by the exact method, and for medium and large farms, a metaheuristic is required for this planning.

## 6. Conclusion

The sugarcane contributes significantly to the economies of many countries. However, there are still great challenges for sugarcane culture such as increase sugarcane productivity. Several studies have been developed aiming to obtain improvement of the genetic base of sugarcane

varieties; increase production of first and second generation ethanol; obtain improvement of the environmental integrated production and recycling management; develop new technologies applied to the sugarcane culture; obtain more efficient machines to planting and harvesting of sugarcane; improve vehicles and improve job qualification and many others. Other researchers from universities have established partnership with private companies in the sugar, ethanol and energy sector, aiming to solve the logistical problems, mainly focused on harvesting logistics.

The transition from manual harvesting to mechanized harvesting promoted many productive gains and reduced losses; on the other hand, the harvesting system demanded a more complex planning, necessitating the development and application of mathematical and computational techniques, aiming to assist managers to make more assertive decisions during this agricultural planning.

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Section 5

# Industrial Applications

Chapter 12

# Sugarcane Bagasse and Cellulose Polymer Composites

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Additional information is available at the end of the chapter

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**Abstract**

Waste recycling has been the main topic of various scientific researches due to environmental management. Renewable agricultural sources such as pineapple leaf, sisal, jute, piassava, coir, and sugarcane bagasse are among agro waste, normally known as biomass, which is recently used for reinforcing polymeric materials. Sugarcane bagasse fiber residues has been extensively investigated and employed as a source of reinforcement of polymers. The major residue is normally burnt for energy supply in the sugar and alcohol industries and as a result, tons of ash is created. The ash contained inorganic components which are valuable for reinforcement in polymeric materials. This chapter reports on the use of sugarcane bagasse, sugarcane bagasse ash (SBA) and its cellulose as reinforcing fillers for polymers.

**Keywords:** sugarcane bagasse ash, reinforcement, energy production, cellulose, sugarcane bagasse

## 1. Introduction

In the past few years, the high utilization of fossil fuels has led to difficulty in recovering petroleum reserves, which has enhanced environmental concerns together with energy security drawbacks [1]. These issues together with global climate change due to greenhouse gas have led researchers to consider alternative fuels based on sustainable bio resources. Agro-energy crops and plant residues are promising low-cost, sustainable biomaterials for biofuel and power generation.

First generation bioethanol has been employed mostly for vehicle fuels which resulted in lowering carbon dioxide ($CO_2$) in comparison to fossil fuels. On contrary, the high demand

for first generation ethanol requires high feedstock production which will lead to food against fuel concerns. The second-generation biofuels becomes the favorite choice since it depends on non-food bio resources such as lignocellulosic. The lignocellulosic materials are relatively inexpensive and available in large quantities. One of the most well-known lignocellulosic materials for second-generation ethanol production is sugarcane bagasse [1, 2].

According to the available literature about 640–660 Mton of sugarcane could generate a total of 28,500 million liters of alcohol, with the aim of producing 45.4% of sugar and 54.6% of alcohol. This would apparently produce 160 Mton of sugarcane bagasse [3]. Generally sugar cane bagasse consists of cellulose (41.0–55.0 wt%), hemicellulose (20.0–27.5 wt%), lignin (18.0–26.3 wt%) and others (~7.0 wt%) attributed to inorganic materials [3–5]. Sugarcane bagasse can be employed for other applications that include extraction of all the constituents (cellulose, hemicellulose and lignin) [4, 5]. Furthermore the sugarcane bagasse ash could be used as raw material for obtaining new type of mortars and concretes [6]. In fact, it has also a potential to partially replace Portland cement [7].

In this chapter we cover all the aspects related to the residues and/or left overs resulting from sugar extraction process. These residues can be used for various applications especially in polymer composite. The high mechanical strength of the sugar bagasse fibers as well as its constituents such as lignin, hemicellulose and cellulose can be added to polymeric matrices to produce multifunctional composite materials. The ashes from the burning of sugar bagasse as source of energy for sugar extraction and alcohol industry can also be used for polymer reinforcement.

## 2. The production of sugarcane bagasse globally (producing countries)

Sugarcane bagasse originates from Asia and can be found mostly in tropical and subtropical climates [1]. For instance, *Saccharum spontaneum* is endemic in the wild from eastern and northern Africa, through the Middle East, to India, China, Taiwan and Malaysia through the pacific to the New Guinea. Globally (mostly in Latin America and Asia), the production of sugarcane bagasse was approximately 1900 million metric in the past 5 years. Brazil is by far the world's largest sugarcane producer with around 740 million ton cane crushed in the 2010/2011 harvest season, which is about 43% of the global production. **Table 1** summarizes the sugarcane crop production in selective countries between years 2009 and 2013. Brazil is also the largest exporter of ethanol, and it is widely expected that Brazil has a large potential for growth in the next decades. At the sugar mills, bagasse has been used to fuel the boilers that supply the thermal and electrical power needed for the internal processes of the sugar mills.

Columbia has been producing sugarcane and palm oil since the early 1900s. Most of Colombia sugarcane bagasse plantations are situated around the Cauca River Valley, and grow the whole with the potential to produce 950,000 $ld^{-1}$ of ethanol from sugarcane juice. There are approximately more than 20,000 registered sugarcane growers regions in South Africa which include the province of KwaZulu-Natal, Mpumalanga and Eastern Cape. The majority of sugarcane

| Country | Year | Average production (million metric ton $yr^{-1}$) | Average annual yield of sugarcane (metric ton $ha^{-1}$) |
|---|---|---|---|
| Brazil | 2013 | 743.0 | 120.0 |
| Mexico | 2012 | 42.5–44.6 | 65.0 |
| Colombia | 2013 | 21.5 | 108.0 |
| Argentina | 2010 | 19.0 | 56.0 |
| Cuba | 2009 | 11.6 | 22.4 |
| India | 2012–2013 | 350.0 | 70.0 |
| Thailand | 2013 | 100.1 | 62.6 |
| China | 2013 | 125.5 | – |
| South Africa | 2013 | 20.3 | – |

**Table 1.** Sugarcane production from different countries between 2009 and 2013 [1, 8–10].

crop is grown in the highest latitude in the country which is in KwaZulu-Natal. India is one of the largest producers of sugar in the world and is the world's second largest producer next to Brazil of sugarcane. Its cane is normally planted throughout 3 seasons in the northwest region of the country. Previously Cuba used to be one of the largest sugar exporters in the world until it was hit by commercial trade blockage 4 decades ago [1, 8–10].

## 3. Physio-chemical properties of sugar bagasse

### 3.1. Fibers

Sugarcane bagasse is a fibrous material obtained as a residue from the sugarcane after crushing to extract the juice. Its stalk is composed of two components *viz.* outer rind and inner pith [11, 12]. The rind consists of strong fibrous structure protecting the inner soft spongy structured material (pith). It contained long finer fibers arranged randomly throughout the stem bound together by lignin and hemicellulose, while the inner component contains small fibers with major part being sucrose. Chemically, sugarcane bagasse composed of cellulose, hemicellulose and lignin [13]. The content of these constituents may vary depending on the growth region and conditions. About 40–50% of dried sugarcane bagasse is cellulose with 25–35% is hemicellulose and 17–20% lignin with some wax 0.8% and ash 2.3% [12, 13]. All these components have similar structure as the constituents of every natural lignocellulosic fibers and, the only difference is their content. In the next subsection only part of sugarcane bagasse and products that can be used as reinforcing fillers of various polymer matrices will be discussed. Moreover, carbonized sugar bagasse can be prepared by alkali treatment followed by burning in the furnace at higher temperature (>500°C) to produce ashes as shown in **Figure 1** [14]. These particles also serve as the most potential reinforcement for various polymeric materials. They appear solid in nature with irregular finer shapes; and it composed mainly of $SiO_2$, $AlO_3$, MgO, and $Fe_2O_3$.

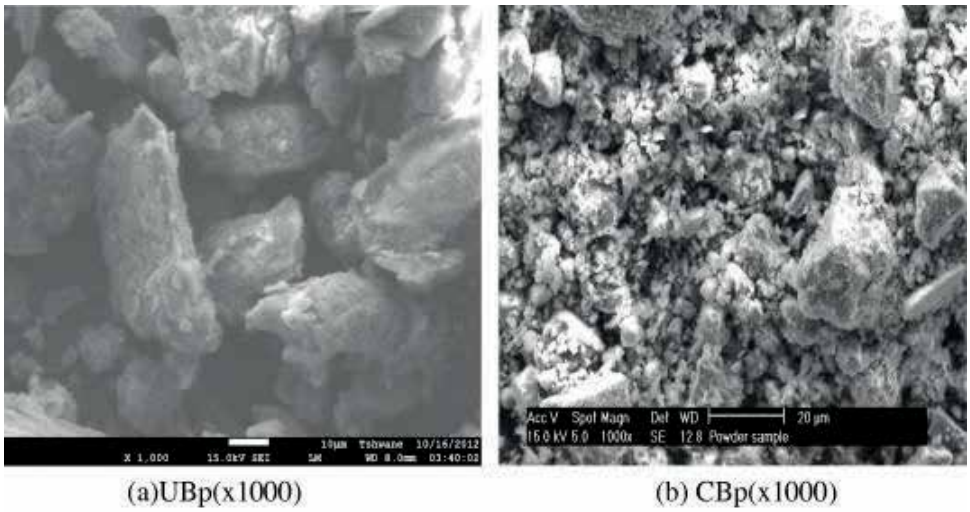


**Figure 1.** SEM images of (a) uncarbonized and (b) carbonized sugar bagasse [14].

### *3.1.1. Cellulose*

Cellulose from sugarcane bagasse can be extracted by using either chemical or mechanical means [15–17]. In some cases both of these (chemical and mechanical) methods are used in order to control the size as well as to improve the purity of the resulting product. A combination of mechanical shearing (or sonication) and controlled acid hydrolysis (or combination of acids) are often used to isolate the cellulose. This kind of cellulose is also known in literature as microfibrillated cellulose (MFC) due to their size diameters ranging from few nanometer to few micrometers, while their length may be above a micron [15–18]. Depending on the time and acid concentration the lateral amorphous region of the MFC can be dissolved to obtain highly crystalline particles. This kind of cellulose particles are known in literature as cellulose nanocrystals (CNC), cellulose nanowhiskers (CNW), cellulose whiskers (CW), microcrystals, or cellulose nanoparticles. These particles have diameters ranges between 5 and 20 nm and the lengths from 20 nm to 1 micron. Tensile strength and/or modulus of these particles and the abundant availability of their source spurred much interest as replacement of engineered reinforcing fillers [19]. The presence of the hydroxyl groups on the surface of these particles offers the advantage for their functionalization [20, 21].

### *3.1.2. Ashes*

Sugarcane industries produce large quantities of sugarcane bagasse (i.e., fibrous residue/left over after sugarcane stalks are crushed to extract their juice) annually which in turn is used in the plant for energy co-generation for sugar processing and/or alcohol production [22–26]. Consequently, the black solid waste produced is collected using a bag house filter as a by-product known as sugarcane bagasse ashes. The collected by-product often consists of fine burnt and coarse unburnt or partially burnt particles. These ashes are non-biodegradable which causes environmental concerns considering their disposal. Some industries adopted

the unfriendly disposal methods such as mixing the ash with water and discarding it into the open field and/or using these ashes as fertilizers. The ashes are composed of highly crystalline phases *viz.* quartz ($SiO_2$), cristobalite ($SiO_2$), potassium carbonate ($K_2CO_3$), calcium phosphate ($Ca_3(PO_4)_2{\cdot}H_2O$), hematite ($Fe_2O_3$), and mullite ($3Al_2O_3{\cdot}2SiO_2$) [24]. The percentage of these constituents varies with crystalline silica being the major constituent (60 to above 80%). The variation is as a result of the growing conditions of the sugarcane bagasse as well as other factors such as soil type, fertilization methods, and soil management. Moreover, the physical and chemical compositions are directly influenced by combustion processes such as combustion temperature and time, cooling duration, ash collection methods and grinding conditions [27]. It is noteworthy mentioning that despite these variations and the reasons given from each study, it can be concluded that these variations comes due to the fact that sugar bagasse is a natural material. The chemical composition can vary as tabulated in **Table 2**. These properties plays major role on the performance of the product manufactured from these ashes. Therefore one of the prerequisite before implementing sugarcane bagasse ashes is to characterize their composition using techniques such as XRD (for crystallography) and EDX (for chemical analysis) as well as to measure their size and/morphology using microscopic techniques such as SEM and other. Loss on ignition (LOI) is also considered to measure the organic matter in the sample or the amount of carbon which reflects extends of ashes combustion. The higher LOI indicates that the amount of unburnt carbon in the ash. The crystalline phase of mullite depends on the ratio of Si/Al of the material. At low Al content the tetragonal structure is obtained which changes to orthorhombic structure at higher Al content. Its nucleation can be accelerated by adding additives, flux or mineralizer. The degree of crystallinity for silica is also dependent on the combustion temperature. If higher temperatures are used during combustion, the more crystalline silica is obtained and for low combustion temperatures the opposite prevails. These changes in combustion affect the specific area and the morphology of the resulting ashes. As mentioned earlier the carbon content can be controlled by these

| | |
|---|---|
| $SiO_2$ | 60–86 |
| $Al_2O_3$ | 1–14 |
| $Fe_2O_3$ | 1–14 |
| $TiO_2$ | 0.2–3 |
| CaO | 2–7 |
| MgO | 0.05–2 |
| MnO | 0.1–0.5 |
| $K_2O$ | 0.2–6 |
| $P_2O_5$ | 0.5–3.5 |
| $SO_3$ | 0.2–2.5 |
| $Na_2O$ | 0.01–0.1 |
| $Cr_2O_3$ | 0.01–0.1 |

**Table 2.** Chemical composition of sugarcane bagasse ashes [24].

changes in the combustion temperatures as it can be obtained at burning temperatures ranging between 400 and 500°C.

## 4. Applications of composites

Biomass residues generated from agro-businesses gained much attention as reinforcement of various polymeric materials due to their inherited properties (i.e., biodegradability and renewability, etc.) and low-cost production. In addition, the consciousness about conservation of the environment has forced the industrial and scientific communities to look for an alternative for the agro-business generated waste. Several biomass residues (i.e., rice husks, maize stalks and sugarcane bagasse) were successfully incorporated into different polymers to improve their mechanical and physical properties. Herein we will discuss the application of sugarcane bagasse as reinforcing filler for various polymeric materials. Different forms of fillers can be generated/produced from sugarcane bagasse waste which include sugarcane bagasse ashes (SBA), fibers (SB), nanocrystals (CNCs), cellulose and micro/nanofibers (MFC or CNF) were incorporated in various polymer matrices such as polypropylene (PP) [28], low density polyethylene (LDPE) [29] polyethylene oxide (PEO) [30], nylon [31] as well as thermosets [32, 33].

### 4.1. Composites processing

It is recognized that the processing technique plays major role on the dispersion of the fillers in the polymer composite materials. There are three widely used methods which include melt compounding, *in situ* polymerization and solution casting. It is of interest to mention that the size of the filler also plays a significant role on the dispersion especially with regard to their processing technique. In most cases micro-fillers (fibers) are preferably prepared *via* melt compounding which is the most conducive method with regard to industrial scale-up process. On the other hand, the nanostructured sugar bagasse-based fillers (e.g., CNCs) are preferably prepared *via in situ* or solution casting which are known to improve their dispersion because these filler are mostly obtained in a solution form which controls their processability. This in turn have forced most of the fabrication methods to concentrate on keeping the dispersed state of the fillers in the solution by adopting either the solution processing technique or polymerization in the presence on the nanostructured fillers.

#### *4.1.1. Melt compounding*

Melt compounding is the most preferable processing technique for industrial scale-up. This technique is usually suitable for highly hydrophobic polymers which normally lead to inhomogeneity of the resulting composite materials. The functionalization/chemical modification of the fillers and/or polymer can be applied to overcome the agglomeration in order to achieve durable and desired properties [14, 34]. The alkali pre-treatment of sugarcane bagasse is often applied to increase roughness of the fibers by removing some of the wax and non-cellulosic substances. It was reported that this kind of treatment was not suitable to improve the interaction/adhesion

and dispersion of the fibers for highly hydrophobic polymer matrices which causes detrimental effect on the properties of the resulting composite material [35].

Compression molding was also used to prepare the SB-based composites [36]. This method, however, resulted in blisters, weak interfacial adhesion and inhomogeneous fiber distribution, regardless of the fiber retreatment.

Melt extrusion followed by melt compression of SB/HDPE composites was studied by Mulinari et al. [37]. The SB cellulose was extracted using sulfuric acid in a reactor followed by surface modification using zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$). In another studies, they used thermo-kinetic mixer followed by compression molding [17, 38]. The modification using $ZrOCl_2 \cdot 8H_2O$ reduced the extent of agglomeration in the composite materials. Moreover, the adhesion between the cellulose and HDPE was improved by surface modification. It was concluded that these processing methods are applicable to produce composites materials using hydrophobic polymers such as HDPE and PP. It worth mentioning that these melt processing methods did not have a significant influence on the extent of the dispersion of the fillers as well as their adhesion. As far as the modification of the filler surface is concerned, it can be concluded that regardless of the type/structure (form) of filler the surface modification can improve the dispersion and adhesion for improved properties.

#### *4.1.2. In situ processing*

The addition of the filler into the precursor (polymer monomer) increases the possibility of good dispersion and interaction between the polymer and filler. Motaung et al. [31] prepared CNC/nylon nanocomposites *via in situ* polymerization. The CNC were added into hexamethylenediamine (i.e., nylon monomer) followed by sonication to enhance the dispersion of the CNCs. Nevertheless, the content of the filler played a major role on the dispersion as well as adhesion. Despite the better dispersion obtained under this processing method, for CNC-based composites this scenario can cause a detrimental effect on the resulting properties of the composite materials. The interwhiskers network formed between the nanocrystals is important to achieve desired properties in CNC/polymer nanocomposites [39, 40].

#### *4.1.3. Solution casting*

Sugarcane bagasse is currently used as a one of the sources of the cellulose nanocrystals (CNCs) for the reinforcing polymers. In these studies the state of dispersion of the CNC in water was maintained by adopting the solution casting method [41, 42]. In this method the nanocrystals are mixed with the polymers in a suitable solvent and allow the solvent to evaporate. Uniform distribution of the nanocrystals within the polymeric material was obtained which can lead to other physical and/or chemical properties. It is also essential to take into account the amount of the nanocrystals incorporated into the polymeric material since the higher the content may result in the agglomeration of the nanocrystals which could cause detrimental effect on the intended application or desired properties [42]. Similar preparation method was utilized in the preparation of SB fibers composites especially for polymers which are soluble in water [30].

*4.1.4. Other processing methods*

Thermosets polymer composites are usually prepared by curing at a temperature depending on the resin-type. The casting of the constituent of the composites onto the steel mold followed by compression molding under certain conditions (pressure, time and/or temperature) influence the properties of the resulting composite material [43–45]. de Sousa et al. [43] studied the effect of pressure on the pre-treated chopped SB-polyester composites. They reported that the combination of all other parameters such as size of the filler, pre-treatment and pressure exerted during molding can be optimized to obtain the desired properties. It is interesting to note that the thermosets have an edge over other polymers due to the fact that it can offer high filler loadings (>65–80 wt%). In addition, the processing temperature is lower when compared to melt mixing and the easy processability. The disadvantage of these composites is that they are not recyclable, and the highly possible alternative is to use them as polymeric fillers or for heat generation. Nevertheless, there has been paradigm shift from synthetic polyesters to a new class of biodegradable resins to overcome the recycling issues [45].

## 5. Mechanical properties

There are several aspects that play a major role on the resulting mechanical properties of the composites materials such as adhesion, the size of the filler, the extent of dispersion. The adhesion and dispersion could also be dependent on the processing technique, the polarity and the surface modification applied on the fillers [14].

Slavutsky and Bertuzzi [41] prepared starch reinforced with sugar bagasse nanocrystals (CNCs) through solution casting. The strong interaction between starch and CNCs due to their chemical structure (polarity) similarity resulted in improved mechanical properties. This was promoted by hydrogen bonding resulting from hydroxyl groups on the surface of CNCs interacting with polymer chains which lead to an increase in Young's modulus (from 112 to 520 MPa) and tensile strength (from ~2.8 to ~17.4 MPa). The chemical treatment on either the CNCs or polymer matrix can also be used to improve the mechanical properties of the ensuing nanocomposites [42]. This is as a result of the crosslinking networks of the polymer which additional strength to the stiffer CNCs or the improvement of the interaction/adhesion between the polymer and the CNCs.

The modification of sugarcane bagasse cellulose with zirconium oxychloride was found to improve the interfacial interaction as well as dispersion which resulted in enhanced mechanical properties [17]. The composites were prepared by melt extrusion with high density polyethylene (HDPE) as polymeric matrix, and the Young's modulus and tensile strength increase respectively from 732 to 1233 MPa and 1.54 to 18.2 MPa. Such increment shows that the mechanical properties the high reinforcing effect of these SB-based fillers can be exploited if the suitable modification is applied. It was reported elsewhere that even the pre-treatment of the sugarcane bagasse fiber with strong acid followed by alkali can improve the interaction between the hydrophobic polymer (polypropylene in this case) and the inherently hydrophilic SB [38]. An increase of 16% in tensile strength and 51% in tensile modulus when compared to pure polymer was obtained.

Most of the SB polymer composites are prepared through melt compounding, thus is often based on the hydrophobic thermoplastics. This results in reduction of the mechanical properties of the resulting composite materials due to lack of adhesion as well as inhomogeneous fiber distribution [36, 37]. Chemical treatment can be utilized to improve the distribution as well as interaction/adhesion between highly hydrophilic SB fibers and hydrophobic thermoplastics [37]. Similarly, the sugarcane bagasse ashes (SBA)-based composites are prepared *via* melt mixing with an additional treatment being applied on either polymeric matrix or ashes to improve the mechanical properties [1, 46]. Since silica has been used as reinforcement of rubbers, the high content of silica in the SBA opens their applicability in rubber composites. Dos Santos et al. [47] reinforced natural rubber with SBA and found that the strong interfacial interaction between the SBA and rubber improved the mechanical properties. A recent study based on the comparison between the commercial silica and SBA reported that it is possible to replace the commercial silica with SBA as rubber reinforcing filler [48]. It was found that the replacement of commercial silica with SBA did not influence the mechanical properties of the composite materials that much.

The effect of NaOH treatment on the SB for the polyester composites was found to be improving the adhesion between the composites' components [45]. The alkali treatment led to finer fibers due to dissolution of the hemicellulose which increased the aspect ratio. A maximum improvement with only 1% NaOH was obtained with 13% in tensile strength, 14% in flexural strength and 13% in impact strength compared to untreated composites. This resulted in better interfacial adhesion between the polyester and NaOH-treated fibers. Other surface treatment of the SB fibers utilized as reinforcement of the thermosets were also studied to improve the interfacial adhesion between the fibers and the polymeric matrix [32, 49]. Despite the general observation of the mechanical properties which increases linearly with increase in fiber content some of these treatment significantly improves the overall performance of the thermosets composites [32, 49]. Vilay et al. [49] pre-treated the SB fibers with NaOH followed by acrylic acid (AA). They reported that the AA treatment improved the tensile strength, Young' modulus, flexural strength, and flexural modulus of the composites when compared to the untreated and NaOH-treated fibers. The elastic modulus was also increased for the treated fibers compared to other with glass transition ($T_g$) shifting to higher temperatures. This could be due to the enhanced interfacial adhesion between the polymer and the filler as confirmed by increase in $T_g$ justify the restriction of polymer chains movement by the reinforcing filler.

A rind and pith component of the SB-based unsaturated polyesters composites was investigated [50]. The flexural strength and flexural modulus were found to increase with fiber content for pith and rind fibers; and impact strength showed similar behavior. The tensile properties were also increased as compared to the unfilled polymeric material. It was, however, found that the rind outperform pith based composites. This was related to the structural difference between the pith and rind. The pith consists of big hollow cavities called lumen reducing bulk density of the fiber and acts as acoustic and thermal insulators. On the other hand, the rind have small size lumens and many finer cellulose fibers. Similar study was conducted elsewhere using poly (vinyl chloride) as matrix [11]. It was also reported that the rind/PVC displayed superior properties (i.e., flexural strength and modulus) when compared to the pith/PVC composites.

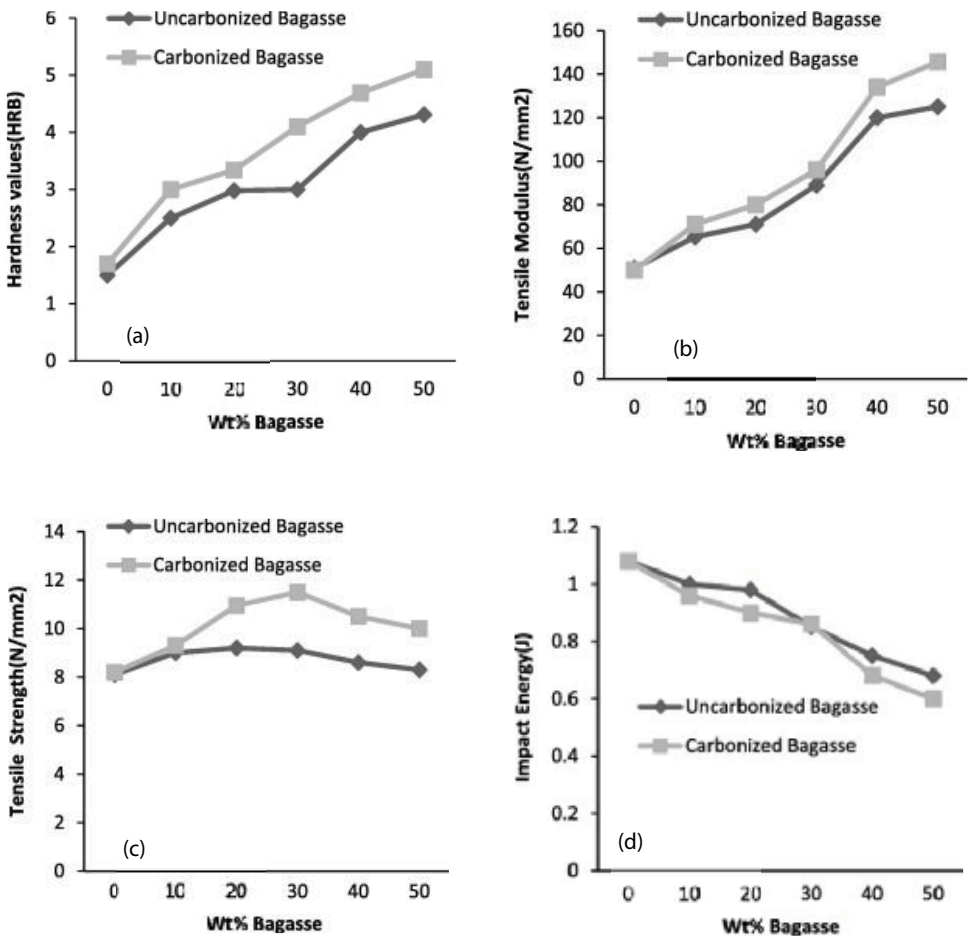


**Figure 2.** Variation of (a) hardness, (b) tensile modulus, (c) tensile strength, and (d) impact energy with wt% bagasse particles [14].

Agunsoye and Aigbodion [14] compared the mechanical properties of the uncarbonized and carbonized bagasse. The hardness was found to increase with an increase in fiber content due to the brittleness of bagasse particles; however the higher values for carbonized particles were associated with their larger surface area (**Figure 2a**). Similar observations were reported for tensile modulus due to the introduction of stiffer bagasse as compared to the polymeric matrix (**Figure 2b**). They observed an increase in tensile strength up to 30 wt% which was attributed to good distribution and dispersion resulting in strong interaction (**Figure 2c**). Above 30 wt%, the decrease was attributed to the physical interaction and immobilization of the polymer matrix by the presence of mechanical restraints. In addition, the decrease in interfacial area with an increase in particles content contributed to reducing the strength. On the other hand the impact strength results showed that the incorporation of these particles reduced the ability of the matrix to absorb energy and thereby reducing toughness. The ability to resist the

impact force was higher for uncarbonized bagasse as compared to carbonized bagasse which was related to the presence of high content of silica adding to the brittleness of the carbonized reinforced composites (**Figure 2d**).

## 6. Water absorption

Water absorption is the most important aspect considering the usage of the fiber polymer composite material in various applications with different environmental conditions. Natural fibers are hydrophilic which lead to their mechanical failure during an application. For example, for a sandwich fiber polymer composites delamination between fiber part and a polymer could ensue as a result of moisture absorption. This is directly dependent on the polymer-type, temperature, humidity, fiber loading, orientation, fiber-matrix adhesion, and permeability of the fibers [35, 50, 51]. On the other hand, surface modification of the fibers may improve the interfacial adhesion between the fibers and the polymer matrix which in turn enhance water absorption resistance. This apparently emanated from the hydrophobicity of the fillers and interaction with the hydroxyl groups on the surface of the fillers, thus decreasing the overall water absorption of the composites. Vilay et al. [49] reported that the treatment of the fibers with acrylic acid (AA) improves the water absorption resistance of the composites. The chemical treatment reportedly reduced the hydroxyl groups which improved adhesion between the fibers and polymeric matrix. The difference between the pith and rind on the water absorption was studied by Lee and Mariatti [50]. The bigger size of lumens in the pith-fibers facilitated the water absorption into the composite material when compared to the rind-based composites.

## 7. Thermal properties

There are two widely used techniques to study the thermal behavior of the natural fiber composites *viz.* thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC). The TGA is usually used to evaluate both the thermal stability as well as the percentage of the fibers in the composites. The thermal stability of the SB-based fillers were studied by several authors to evaluate the effect of the extraction processes and surface modification [15, 16, 52]. The degradation steps of the fillers give an idea of the resulting product after extraction process. Similarly, the endotherms from DSC often shows the steps involves during heating process such as evaporation of water or moisture below 100°C.

Surface modification of the fibers can also change their thermal degradation behavior [32]. In the case of furfural as surface modification, it interacts mainly with lignin components (i.e., phenolic syringyl and guaiacyl) which alter the thermal degradation behavior of the fibers especially the step associated with lignin [32]. The alkali treatment improve the thermal stability of the fibers due to the removal of thermally unstable constituents of the fibers (i.e., hemicellulose, and wax). On the other hand the acid hydrolysis during the extraction of cellulose fibers (MFC) or cellulose nanocrystals (CNCs) introduces some thermally labile groups

on the surface of the fibers which results in reduction of thermal stability. In addition these harsh conditions may reduce the crystallinity and molecular weight of the cellulose which also contribute to the reduction of thermal stability.

## 8. Conclusions and remarks

The abundant availability of sugarcane bagasse offers an alternative toward the engineered fillers as well as the seasonal natural fibers as reinforcement of polymers for various applications. It can be argued that the hydrophilic character of sugar bagasse adversely affect the properties of the composite materials. Most of the studies based on surface modification of the fibers proved that these fibers can be applied in various fields such as aerospace, construction and automotive if the suitable surface modifier is applied. However, these modifications must be applied in such a way that they do not influence other properties especially for the fibers which are very sensitive toward harsh conditions which may adversely affect their durability and versatility. The inherited properties such as biodegradability and renewability has to be considered during the production of the composite materials. All processing techniques have their benefits and limitations. For example, the CNCs-based composites are preferably prepared *via* solution casting and *in situ* polymerization to obtain highly homogeneous distribution of the filler and interfacial adhesion without surface modification. In addition the low yield obtained from the extraction process *viz.* acid hydrolysis limit their application in melt compounding where large quantities of the filler are required.

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Chapter 13

# Sugar Versatility—Chemical and Bioprocessing of Many Phytobiomass Polysaccharides Using a Milder Hydrolytic Catalyst: Diluted Thermopressurized Phosphoric Acid

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Additional information is available at the end of the chapter

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**Abstract**

Phytobiomasses, given the qualitative and quantitative dominance of polysaccharides, are a dominant wealth available in nature. Cellulose and hemicelluloses from softwoods, hardwoods and grasses, starch from tubercles and roots, pectins from fruits and gums from some seeds may be explored as such or following acid or alkaline pretreatments as well enzymatic deconstruction, and even simple chemical derivatization toward more added-value products. A general view in the chemistry of these valuable polymers is here broached, following a sharper focus on acid pretreatments for L(h)C—ligno(hemi) cellulosic materials from sugarcane and other feedstocks. Our particular experience using a gentler proton donor but keeping very advantageous aspects for polysaccharide chemo/biotechnological processing—thermopressurized diluted phosphoric acid (oPA)—is presented with a more detailed description as a result of its validity for the hydrolytic deconstruction of hemicelluloses—heteroxylans and heteromannans, cassava starch, dahlia inulin and mixed glucans from microalgae cell walls. The opportunity of NOs—nutraceutical oligosacchrides—generation from these particular glycopolymers is also shortly commented.

**Keywords:** phosphoric acid, polysaccharides, sugarcane, ligno(hemi)cellulose, starch

## 1. Introduction

It is intriguing remarkable the metabolomics of some biomass such as pentose or hexose derivatives. The 2-deoxyribose is the only carbohydrate selected to integrate the most important macromolecule of living organisms; DNA or deoxyribonucleic acid. It is also a component of riboflavin or vitamin B2 and the energetic main coins, ATP and similar coenzyme structures. However, the occurrence in nature of its mother molecule, ribose, is not so common. Crotonoside or isoguanosine, an exotic ribonucleotide is present in croton bean (*Croton tiglium*) and in the wings of the butterfly *Prioneris thestylis* [1]. Its beneficial action against tumors was demonstrated. Ribosyl units in polysaccharides are quite rare and one example is the capsular polymers from some pathogenic bacteria. These short comments lash out us to a pertinent question: why deoxyribose-built DNA and ribose-built RNA (mRNA, rRNA and tRNA) molecules? The answer is rather complex considering the consequences from this single difference: vital metabolic roles, chemical stability, rate of degradability facing ribonucleases and deoxyribonucleases, etc.

Conversely, glucose is the most widespread carbohydrate and the main energetic fuel in any organism besides the homocomponent in the most common nature polysaccharides such as starch (plants, molds, bacteria and microalgae) and glycogen (mammals); while 2-deoxyglucose inhibits organisms growth blocking glycolysis, although some controversial benefits for the treatment of epilepsy and also has been proposed as therapeutic tool for some types of cancer [2].

Monosaccharides are the major source of fuel for cell metabolism, bioconversion processes and structural materials [3–5]. D-glucose is the most universalized carbohydrate occurring in the nature under polymerized forms. In these natural polymers, the dominant glycosidic linkage is 1,4 connecting the anhydropyranose residues, as $\alpha$ or $\beta$ anomeric configurations as is the respective cases of cellulose and starch. However, besides these examples of homopolyglucoses, glucopyranosyl units may integrate the whole structure of important heteropolymers like glucogalactomannans in conifers or softwood hemicelluloses. Also, worth of mention is that both ionic forms of glucose—glucuronic acid and glucosamine—make portion of other important natural polymers such as acidic xylans from hardwoods and chitin from marine crustacean and even aquatic mold cell walls.

Disaccharides are produced naturally and in abundance in plants such as sugar beet and sugarcane. Sugarcane comprises several grass species of the genus *Saccharum* chosen as feedstock in tropical and subtropical countries to produce sucrose which in turn can be fermented to produce bioethanol, notably by the Brazilian sugarcane industry. The remaining sugarcane biomass (bagasse and straw) can be burnt to electricity production, left in the field (straw) for agronomic purposes, or, more recently, applied in industrial scale for the production of bioethanol after deconstruction by a pretreatment of choice followed by enzymatic hydrolysis and a fermentation step.

Polysaccharides, as natural polymers, are by far the most renewable resource in the Earth [6]. They are the products of a natural carbon-capture process, namely photosynthesis, that

follow further biosynthetic modifications to carry out various specific functions in plants and other organisms. Examples include structural polymers such as cellulose, chitin, pectin and storage polysaccharides such as starch and inulin. Because of their huge structural diversity (e.g. pentoses, hexoses, aldo- and keto-sugars, deoxi-derivatives like fucose and rhamnose, D and L-configurations, other glycosidic type linkages other than 1,4) and functional diversity, polysaccharides and monosaccharides are expected to play a progressive role in industry, either in their native or chemically modified forms. As some polysaccharides (such as cellulose, starch and chitin) are produced on a very large scale in nature, the interest in their hydrolytically or non-hydrolytically processing is strongly associated with a variety of applications in the food, paper, pharmaceutical, cosmetic and biofuel industries.

As one stringent example, considering how much purified cellulose (e.g. from textile weaving yarns) is simply discarded: a large-size textile factory in a daily operation with cotton treads can accumulate as much as >1.5 ton of cotton dust waste (CDW) around the loom machines. The collected cellulosic residue may be just burned to generate vapor and additional energy supply thus aggravating the local greenhouse environmental problem. We are partially alleviating this situation by, through one pot reaction, transforming the mercerized cellulose residue in ionic forms (CDW-Carboxymethyl and CDW-diethylaminoethyl positively charged—DEAE+) and efficiently utilizing these insoluble ion exchange matrices to, respectively, sequester/remediate a large volume of residual cationic and anionic dyes from the factory wastewater [7, 8].

This chapter will cover two major polysaccharides, cellulose and starch, and their deconstruction from different substrates with emphasis to the advantages and wide applicability of aqueous moderately thermopressurized phosphoric acid pretreatment for bioethanol production as for other applications such as oligosaccharides with nutraceutical properties.

## 2. Brazilian sugarcane industry in brief and environmental issues

Brazilian agribusiness is the strongest arm of its whole economy. The contribution from sugarcane business and derived products is outstanding for local consumption and exportation, financial incomes and, in our view, overcoming concurrent activities such as soy, corn and coffee commodities. About 1 $m^3$ of sugarcane juice may contain around 200 kg of sucrose, easily splitable into their valuable counterparts: glucose and fructose. These monosaccharides are equally and promptly hydrolyzed into the same monosaccharides by *Saccharomyces* spp. hyperactive yeast cell wall-bound invertase, capable to quickly pave the metabolic pathway of this precious aldo- and keto-aldoses to ethanol and other useful fuels.

Brazil, secondly followed by India, is the world leading processing sugarcane to first generation ethanol, table sugar and other goods as corroborated by its huge year-crop (2016–2017) in the range of 657.2 million tons with a final production of 11 billion liters of anhydrous ethanol and around 39 million ton of table sugar (sucrose) [9]. It is a good time to focus on the Brazilian sustainability scenario and actors: economically feasibility, environmentally correctness and

social fairness. There are gladness and sadness criticisms from both sugar mills entrepreneurs and capitalism lovers versus poorer workers at cane plantations, respectively. Let us emphasize the thoughts and opinions from the closer national teacher and researcher on environmental sciences, recalled his graduation title as a social scientist, too—Prof. Dr. Valdir Fernandes [10]. His comments and Strengths, Weaknesses Opportunities, Threats (SWOT) guidelines were built in a partnership with other three other publication colleagues and was summarized below:

(a) Brazil is committed from many decades ago with sugarcane ethanol as a mandatory surrogate for petrochemical derived fuels; (b) given the huge figures for production and processing, when examining the sustainability, it is necessary to build tools which allow to assess an integrated conception of the sugarcane matter, prospects, goals and subjects, about everything to help and to influence decision-makers to establish public policies for a sustainable development; (c) the complexity may be captured from economic and social indicators without no reduction in the significance of each system component; (d) taking the State of São Paulo—the major Brazilian producer and more developed state federation unit—and the trustable indicators raised by its Environmental Secretariat—sounded as one pertinent strategy for the current evaluation; (e) water supply and its quality regarding environmental implications is a valuable cornerstone; (f) the evaluation of environmental indicators encompasses the application of extensive interviews allied to experts workshop pointing out to a set of benchmarks; in the present case, 16 respectable experts were involved. These interviews established three main focus: water, soil and atmosphere. Each focus considering, respectively, 11, 12 and 2 relevant aspects/opinions input. As illustrative examples, reduce availability, oxygen-deprivation as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) parameters, eutrophication of surface sheets by NPK and respective leaching intensity in the case of water, loss of soil nutrients nitrification and acidification by low-pH vinasse, microbiota flora reduction for the soil focus and some possibly unchanged/unchangeable indicators such as photochemical formation of tropospheric ozone and atmospheric acidification (permanent greenhouse gases release). Discussions and conclusions drawn for other mentioned topics in water and soil derived from the expert's team suggested opinions and additions. A strengths /weaknesses/opportunities and threats, namely, a SWOT analysis was built. (g) conclusively, a better guide for the people taking decisions—sugarcane industrial managers, union leaders, politicians, governmental authorities in agricultural, health, economic and social fields—all them committed with the whole society benefits on safety, welfare and progress—is to consider and refine, *inter allia*, environmental indicators to feed the discussion and legal decisions to support the so needed sustainability in the giant sugarcane business.

To the authors understanding this remarkable contribution of this environmentally proactive scientists quartet from USP—University of São Paulo, UTFPR—Federal Technological University of Paraná and UFPE—Federal University of Pernambuco (prudly and proudly Brazilian scientists!) deserves a complete reading of their corresponding 27 pages full report for any reader interested in the profits and negative implications of any giant agribusiness as well as other related industrial and highly polluting factories on commodities (e.g., pulp/paper, timber/saw mills).

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## 3. Structure of lignohemicellulosic biomass

Cellulosic materials are the most abundant renewable polymer resource available in nature as the main component of plant cell walls, which in turn is subdivided into primary wall and secondary parts. Unlike other homopolysaccharides encountered anywhere, cellulose occurs in close association with hemicelluloses and lignin, then named ligno(hemi)cellulose or shortly, L(h)C (**Figure 1**). Together, these three biomolecules are the main components of plant biomasses corresponding, respectively, as 40–60% for cellulose, 20–40% for hemicelluloses and 10–25% for lignin in any L(h)C biomass [11]. The distribution of cellulose, hemicelluloses and lignin varies considerably among cell wall layers. L(h)C biomass also can contain some pectin and xyloglucan along with minor amounts of minerals (ash) and various other compounds, which are called extractives.

Exceptions for this statement seldom occur in the plant kingdom but is the case of cotton caps and kapok ripen fruits where cellulose fibers are almost pure, meaning free of hemicellulose and lignin. Mention to some polysaccharide bacterial anabolism is mandatory here: some species of acetogenic bacteria, specially species such as *Gluconacetobacter xylinus*, formerly known as *Acetobacter xylinum* and since reclassified as *Komagataeibacter xylinus* [12], biosynthesize effectively pure cellulose ribbons of special architecture as soft biofilm gels. There are now a plenty of medical and other biotechnological applications for this noble cellulose occurrence and intensive production.

Southeastern Asian countries (Thailand, Malaysia, the Philippines and Indonesia) consume it as appreciated food known as "nata-de-coco". We have been consolidating other biotech products, one of them its covalently died derivative (Remazol Brilliant Blue R (RBB)-bacterial cellulose) for cellulolytic enzymes detection and measuring [13], following our pioneering

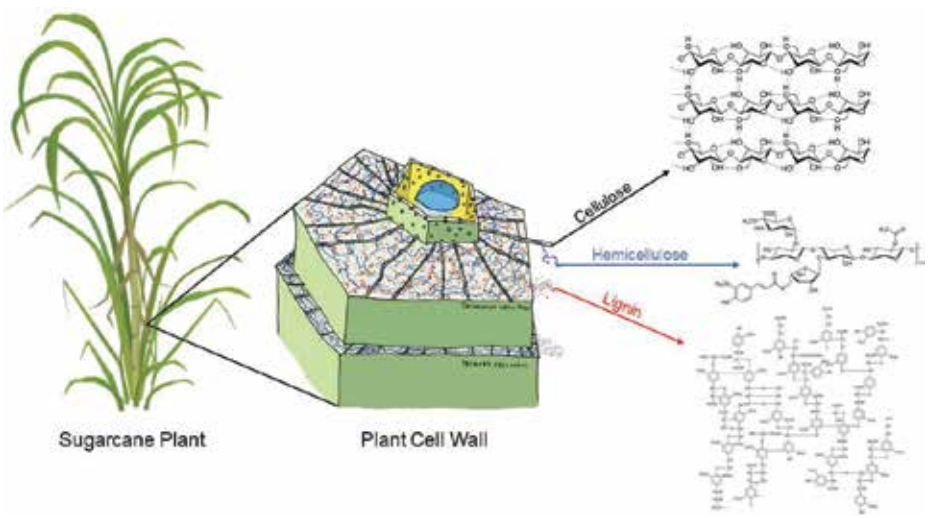


**Figure 1.** General ligno(hemi)cellulose structure of the plant cell wall.

report of its application just after a quick cleanliness for entrapped cells (although known as Generally Recognized as Safe-GRAS bacterium) as a temporary skin substitute in the case of human skin burns and other dermal injuries [14].

Cellulose consists of a collection of linear chains of β-(1,4)-linked D-glucopyranosyl units. L(h) C biomass include agricultural and agroindustrial residues (cane bagasse, cereal straws, corn-stover, cobs or husk and similar polysaccharide-rich materials); wood materials (branches, bark, stumps, wood wastes from sawmills and paper mills) and dedicated energy crops (*Miscanthus* sp., switchgrass, etc.) including energy cane, a hybrid lineage of sugarcane that has been bred and selected for fiber production over sucrose production. In Brazil, pioneer hybrids of energy cane were produced by CANAVIALIS, a private sugarcane breeding company that obtained 138% more total biomass (green matter) per area than a good conventional sugarcane variety and 235% more fiber [15].

The cellulose chains are packed in layers that interact with each other by van der Waals forces with intramolecular and intermolecular hydrogen bonds to form microfibrils [16, 17]. Because of these interactions, cellulose has a recalcitrant crystalline nature that makes it generally resistant to degradation by any mold or bacterial cellulose complexes (endoglucanases + cellobiohydrolases I and II and β-glucosidases). However, some reports have shown that a class of oxidative enzymes, the lytic polysaccharide monoxygenases, have the capacity to degrade recalcitrant crystalline cell wall components, including cellulose [18, 19]. This is, undoubtly, a remarkable progress in biochemical technology.

Hemicelluloses are a diverse group of polysaccharides generally characterized by having a β-(1,4)-linked sugar backbone with the main function to reinforce the cell wall by interaction with cellulose and lignin. In xylans (angiosperms or hardwood and grasses), mannans (conifers and hardwoods) and xyloglucans (predominant in the primary bed of dicot and monocot/non-gramineous monocot cell walls), the backbone sugars are β-1,4-D-Xyl, β-1,4-D-Man, and β-1,4-D-Glc, respectively, while in glucomannans, the backbone comprises of randomly distributed β-1,4-D-Man > β-1,4- D-Glc units [20]. Xylans are the major constituent in secondary plant cell wall comprising a backbone of repeating β-(1,4)-D-Xyl residues most often substituted by L-arabinosyl (Ara*f*) and D-glucuronic acid (GlcpA)/4-O-Methyl-D-glucuronic residues. O-acetyl substituents are also present in the main xylopyranosyl units.

The L-arabinofuranosyl residues can contain ferulic acid groups esterified to the O-5 position of the carboxyl group which in turn can be oxidatively cross-linked to lignin incorporating xylans into the lignin reinforcing even more the network [21]. This feature particularly explains why rye bread hardens as compared with the softer wheat bread. Softwoods and hardwoods can have different hemicellulose content [22]. Hardwood hemicelluloses are composed typically by highly acetylated heteroxylans (4-O-methyl glucuronoxylans) with low amounts glucomannans. Instead, softwoods are common in the presence of partly acetylated galactoglucomannans and glucomannans with xylans corresponding to a minor fraction of their hemicellulose content [22].

Lignin is a phenolic polymer mainly deposited in secondary cell wall coating cellulose and generally combined with hemicelluloses, and built up almost entirely an intervening layer

| L(h)C feedstocks | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|
| Sugarcane bagasse | 42 | 25 | 20 |
| Hardwood | 40-55 | 24-40 | 18-25 |
| Softwood | 45-50 | 25-35 | 25-35 |
| Corn stover | 38 | 26 | 19 |
| Corn cobs | 45 | 35 | 15 |
| Rice straw | 32 | 24 | 18 |
| Wheat straw | 29-35 | 26-32 | 16-21 |
| Grasses | 25-40 | 25-50 | 10-30 |

**Table 1.** Biomass composition in common L(h)C feedstocks (source: Adapted from [24]).

called middle lamella that acts like a cementing agent biding primary cell walls together. Lignin also provides rigidity, enhances mechanical strength, reinforce vascular cells and acts as a pathogen and water-impermeable barrier for the plant tissue [22]. Lignin is built from monolignols based on three monomeric precursors (coniferyl, sinapyl and *p*-coumaryl alcohols) which appear to be incorporated into the lignin polymer in a non-predictable way [23].

It is worth to mention that oppositely to hemicelluloses (in fact always heteropolysaccharides) with a plenty of secondary substituents and also displaying a covalent connection with part of lignin, cellulose is a pure homopoly-*β*-glucan. Its interaction with other polymers are solely based on physical rather than chemical linkages.

A determining factor when selecting biomass for biochemical processing to recover their respective sugars is the different physico-chemical properties of various L(h)C materials, what is based, at first glance, in their major chemical composition. **Table 1** presents how L(h)C can be diverse depending on the origin and part of the plant. As the enzymatic, or even chemical hydrolysis of cellulose is greater than that of lignin, the complete conversion of the carbon-containing plant material present as cellulose is greater for plants with a lower proportion of lignin.

## 4. L(h)C biomass deconstruction to sugars

L(h)C biomass, as said before, can be obtained at relatively low cost in different forms, representing a potential sugar source for the fermentative production of renewable fuels as well as other materials in modern biorefineries. Most of these potential applications rely on the predominant cellulose fraction susceptibility to enzymatic hydrolysis/acid hydrolyses or other structural changes.

However, the intrinsic nature of L(h)C materials is completely different from that found in starch. Starch granule serve as a temporary energy storage polymer with glycosidic linkages that can be readily hydrolyzed to supply glucose for germination and plant growth.

Conversely, L(h)C has been projected by nature to function as a resistant structural material of carbohydrates and lignin that resist assault on cellulose through enzymatic deconstruction from a vast hydrolytic and/or oxidative enzymatic machinery secreted by various microorganisms, be fungal or bacterial. Besides to the protection conferred to cellulose by lignin and hemicellulose, factors such as cellulose crystallinity, low surface availability cellulolytic enzymes and degree of acetylation of hemicellulose prevent or hinder the whole polysaccharide deconstruction by microorganisms [25].

Therefore, more aggressive chemical and/or physical environments are required for the dismantlement of the L(h)C matrix, reducing the cellulose crystallinity, and increasing the porosity to a better accessibility to cellulose complex and more importantly, removing the hemicellulose and lignin barriers, which can be addressed to other technological goods. By doing this, the biomass become suitable for the production of second generation biofuels as ethanol, butanol, ABE (Acetone-butanol-ethanol) mix and other compounds like lactic acid, which can serve as a feedstock for the production of polylactic acid (PLA) to replace the petrochemical packaging materials such as polyethylene terephthalate (PET). In the case of cellulosic ethanol production four main steps must be taken in account: (1) biomass pretreatment; (2) enzymatic hydrolysis; (3) monosaccharide fermentation and (4) ethanol distillation.

There are several conditions for the selection of an appropriate L(h)C pretreatment (pT) method: (1) this should avoid the excessive size reduction of biomass particles, (2) preferably hemicellulose portion must be preserved as such (by alkaline pT), (3) converted do free xylose or xylo-oligosaccharides (acid pT) minimizing in this second case, the co-generation of degradation products like furfuraldehyde, a known inhibitor to microbial growth, (4) reduce the energy requirements, (5) explore a low-cost pT catalyst and/or inexpensive catalyst recycle and (6) preferably regenerating of any form of high-value lignin co-product [24].

Pretreatment can be performed by biological, physical and chemical processes and even combination of them. However, there are several possible combinations or operation modes. The choice of a particular method has to be based on a number of considerations, for example, which biomass will be used, the organism used for fermentation of the released sugars, and the costs implied. Common to all of these methods is that the L(h)C materials must be first mechanically pretreated (ground, chipped or milled) to increase the surface area.

In biological pretreatments, microorganism such as brown, white and soft rot fungi are used for degradation of lignin and hemicelluloses from biomass [26]. The efficiency of the process generally depends on the action of lignin-degrading enzymes such as peroxidases—lignin peroxidase (E.C. 1.11.1.7), manganese peroxidase (E.C. 1.11.1.7) and laccases (E.C. 1.10.3.2), generally copper-containing oxidases produced mainly by basidiomycetes. Supplementation with accessory enzymes like hemicellulases (endo/exoxylanases, $\beta$-D-xylosidases and $\alpha$-L-arabinofuranosidase) increase hydrolysis yields but also enzyme costs and dosages [27]. Although eco-friendly and without production of inhibitory compounds, biological pretreatment is not suitable for a pilot scale process mainly due energy demand and to the long incubation time for effective delignification [28]. However, biological pretreatment was found to be more effective if it is combined with another chemical or physical pretreatment [29].

Dilute and hot aqueous acid pretreatment (e.g., $H_2SO_4$, $H_3PO_4$ and organic acids) is the most widely employed method on industrial scale [24]. Acid pretreatment is efficient in promoting hemicellulose removal where a rich C5 liquid fraction is generated, leaving a solid material with high content in cellulose and less lignin, being the residual cellulose fraction more stable in the acidic regime due to its crystalline structure. This pretreated material can be further submitted to enzymatic hydrolysis with an increased cellulase accessibility. Two general approaches in acid pT can be applied; high temperature (above 180°C) during less residence time (1–5 min) and lower temperature (<120°C) for long duration (30–90 min), respectively [24]. The most conventional commercially used acid is dilute sulfuric acid, mainly to its low cost. However, use of mainly strong acids such as sulfuric, hydrochloric and nitric acids, even diluted, presents various drawbacks. These are mainly related with an increased production of inhibitory compounds (furans like HydroxyMethylFurfuraldehyde (HMF) or its progressive degradation species such as levulinic and formic acids if more parameters severity is applied) and corrosion of reaction vessels [24, 25, 30]. Therefore, various other acids have been used to circumvent the harsh conditions imposed by strong acids, such as maleic acid [31], fumaric acid [31], oxalic acid [32] and, in our repetitive experiences, phosphoric acid, that will be discussed later in the chapter.

In the alkaline pretreatments, L(h)C biomass is treated with alkali, normally sodium hydroxide or lime, $Ca(OH)_2$, at normal temperature and pressure. The main advantage is the efficiency in lignin removal [24, 33, 34]. It also demands relatively low capital costs, allows lower inhibitors formation and ensures high glucose yields in the subsequent enzymatic hydrolysis step, besides the possibility of a better lignin recovery by co-addition of air or oxygen. In the case of lime use, the mild catalyst may be recoverable injecting $CO_2$ in the liquefied alkaline stream [33, 35]. Despite the inexpensiveness of lime and other hydroxides are inexpensive, downstream processing costs are high because the process requires a large quantity of water for the appropriate washing of the residual cellulose [24].

Physico-chemical pretreatments include steam explosion pretreatment which is one of the most used methods for pretreatment of L(h)C biomass. In this approach, biomass is submitted to a high pressure (0.7–4.8 MPa) with saturated steam at high temperatures between 160 and 260°C for a few seconds followed by an explosive decompression, that cause separation of the fibers [24, 33, 36]. It can be carried out with or without (autohydrolysis) addition of an acid catalyst [37]. In autohydrolysis also named solvolysis, the high temperatures (160–250°C), an endogenous catalyst, acetic acid, is delivered from the O-Acetylated xylan and catalyzes the break of part of the xylo-backbone of hemicellulose thus producing of xylooligosacchrides along with some free D-xylose. The few side chain L-arabinofuranose substituents are completely released since their native $\alpha$-configuration (allied to the furanosyl ring) are more prone to any acid hydrolysis [36]. Any alkaline pT can be performed with high total solids, providing higher yields, and a cellulose fraction with improved accessibility to enzymatic hydrolysis [38]. Some drawbacks of steam explosion pretreatment are the partial degradation of hemicelluloses, production of fermentation inhibitors (e.g. aromatic compounds, furfural and HMF) demanding a washing step for detoxification of the pretreated biomass [39, 40].

Most of the demonstration plants installed worldwide aiming cellulosic ethanol production is based on steam explosion pretreatment, or its variations. One example is POET-DSM Advanced Biofuels that constructed a cellulosic biorefinery alongside the POET Biorefining - Emmetsburg plant. The company contracted ANDRITZ Inc. to supply a two-step biomass treatment process that includes a vertical reactor and a continuous steam explosion (SE) technology to pretreat corn residues (stalks, husks, leaves and cob). In Brazil, GranBio began operations in 2014 with 22 million gallons per year cellulosic ethanol facility, Bioflex 1, using Beta Renewables' PROESA pretreatment process, Novozymes' cellulase enzymes, and DSM's yeasts. In PROESA technology plant, the biomass is pretreated with steam (high temperature and pressure) without chemical addition followed by enzymatic hydrolysis (viscosity reduction and hydrolysis). Some companies have reported difficulties regarding biomass feeding/transport and high degree of equipment wear due to the frictional effect of abrasive materials present in biomass [41]; signaling that this kind of pretreatment still remains as a challenge.

Other important pretreatments comprise ammonia freeze explosion (AFEX) [42], liquid hot water [43], organosolv and ionic liquids [44], the last one implying in higher costs despite the alternative of catalyst recycling. Also, new pretreatment technologies are constantly being developed, such as sub-/supercritical water [45] and supercritical carbon dioxide [46].

### 4.1. Thermopressurized aqueous phosphoric acid pretreatment for partial or total depolymerization of L(h)C biomass

Thermopressurized aqueous phosphoric (oPA) as pretreatment of L(h)C from different biomasses followed by enzymatic hydrolysis is an efficient approach to further provide free glucose from residual cellulose and immediate free xylose, mannose and the other minor pentose (L-arabinose) and some hexose from the hemicelluloses portion. As phosphoric acid have a higher pKa value than strong acids (pKa: 2.1 against pKa: −3 from $H_2SO_4$, pKa: −7 from HCl and pKa: −1.3 from $HNO_3$) it effectively more attractive appeal due to the generation of less carbohydrate dehydration than stronger acids, being possible to carry out the pretreatment of the L(h)C substrate over a wide variety of temperature and pH values. Conversely, the past drawback of phosphoric acid being more expensive than sulfuric acid is now under overcoming considering the aggressive entry of China in the production of several commodities including oPA. A detail, however, is to be taken in account: the moisture content of bulk mineral acids, ranging from 2 to 4, 15, 35 and 63%, respectively for sulfuric, phosphoric, nitric and hydrochloric acids. Nitric acid, due the oxidant action and threat to DNA, has been never considered as a biomass pretreatment. Hydrochloric acid and its toxic fumes offer more serious risk for the labor. Sulfuric acid is a risk for storing, serious burns even by short contact and faster equipment corrosion. So, *inter allia*, we historically from 1980 till nowadays, have been elected phosphoric acid, a gentler proton donor, for all our polysaccharide targets: chronologically from sugar and sorghum bagasses, cassava starch, dahlia tubercles inulin and more recently for microalgae cell wall polysaccharides for the special prospect of nutraceutical oligosaccharides preparation. Except for inulin, the most labile substrate, given its unusual $\beta$-1,2-furanofructoside linkage (when 10–30 min heating at 70–80°C in open vessels is

enough to produce the precious FOS—FructoOligoSacchrides), xylan, mannan and microalgal glucans are pretreated under more severe parameters (thermopressurized reactor) in the range of 2 atm (120°C) to 15.5 (200°C) for a short peaking time (1–2 min) but always keeping the effective oPA concentration with a narrow pH 3.0–1.5 range and more often, at pH 2 or its pKa value (2.1).

Almost four decades ago, initial studies with L(h)C biomass (rye grass straw) treated with phosphoric acid was carried out to verify the amount of sugar and yeast fermentability after treatments with various concentrations of $H_2SO_4$, HCl and $H_3PO_4$ aiming to study the feed acceptability by rodents [47]. They have shown that both fermentability (after neutralization with ammonia) and rodent palatability were highest when the straw was treated with a combination of 0.23 N HCl and 0.15 N $H_3PO_4$ (30 min at 121°C), which produced 0.25 g of sugar per g of straw. They also verified that if straw were treated with higher concentrations (>0.5 N) of $H_2SO_4$ or HCl, yeast yield declined probably due to the higher concentration of toxic degradation products of monomeric sugars, such as furfural and HMF.

Prof J.D. Fontana's group at LQBB—Biomasses Chemo/Biotechnological Laboratory formerly at UFPR (Federal University of Paraná) and now at UTFPR (Federal Technological University of Parana, Curitiba-PR, Brazil) has been consolidating for a long time the phosphoric pretreatment technology using very diluted $H_3PO_4$, alone and under moderated thermopressurization. An initial study focused on the pretreatment of sugarcane and sorghum bagasses with $H_3PO_4$ for the production of bioethanol [48]. The main results from this pretreatment using optimized conditions (0.065% v/v $H_3PO_4$ and 200°C, 3 atm, during 2 min) was a complete or partial hydrolysis of hemicellulose fraction to xylose>xylo-oligosaccharides>arabinose depending on the variation of the severity parameters just before mentioned. Moreover, it was observed improved fermentation of the solubilized pentoses to ethanol and acetic acid by *Pachysolen tannophilus* and to ethanol by *Fusarium oxysporum*. *Pachysolen tannophilus* was the first yeast shown to be capable to convert xylose directly to ethanol under anaerobic conditions (with the concomitant production of xylitol and acetic acid) [49], while *F. oxysporum* is one of the few fungal species reported to ferment plant carbohydrate polymers to ethanol in just one-step process [50]. It was observed that fermentation capability was related to lignin solubilization followed by its removal using ethyl acetate or activated charcoal. Most importantly, phosphoric acid pretreatment on sugarcane and sorghum bagasses allowed almost complete conversion of cellulose to glucose using commercial cellulases produced at that time by Biobras (BIOFERM, Brazil), a Brazilian company that operated a 2G ethanol plant in the end of 1970s and was further acquired by Novo Nordisk's in 2003.

Interestingly, even past >30 years, our oPA technology for sugarcane processing has been used with industrial proposals, as, for example, in the CANEBIOFUEL (Conversion of sugar cane biomass into ethanol) Project, that was funded by the European Commission (FP7-Energy) which planned to obtain a deeper knowledge and a scientific and technological platform for converting sugarcane biomass into fermentable sugars. The project concluded that, in general, lower severity during pretreatment, with lower temperatures and shorter times, result in better glucose yield than the opposite. Primarily based on ease of enzyme hydrolysis it was also found that $H_3PO_4$ is superior to $H_2SO_4$ for the acid catalyzed pretreatment. However,

some Brazilian partners in this project applied steam explosion to the pretreatment of sugarcane biomass with almost exactly the same kinetic conditions of our oPA treatment [48]., omitting, unfortunately and consciously, to mention our pioneering publication, as it is completely clear from their below report at Italy.

> *"Steam explosion of cane bagasse using phosphoric acid catalysis", IBS2010 – 14th Intl. Biotechnology Symposium and Exhibition, Palacongressi, Rimini, Italy; 14–18 Sept, 2010."*

In our another work, aqueous $H_3PO_4$ was used to increase the nutritional value of sugar cane bagasse for cattle feeding [51]. Enhanced ruminal degradability (almost 70%) was obtained by adding 2.9% (w/w) in comparison to 60% achieved with solvolysis with water (197°C,13.5 atm, 4:1 w/w of water). Furthermore, $H_3PO_4$ generates less carbohydrate dehydration and does not have to be washed out prior to fermentation because phosphate can act as an important micronutrient, after partial neutralization with ammonia, for the subsequent fermentation step [51, 52].

Steam treatment of sugarcane bagasse with a low level of phosphoric acid (1% of bagasse dry weight) at elevated temperatures (160–190°C) during 10 min resulted in a total sugar yield ranging from 215 to 299 g/kg bagasse (untreated dry weight) and lower levels of products from sugar degradation (furans and organic acids) in all treatment temperatures (140–190°C) as compared to sulfuric acid [53]. Hemicellulose hydrolysates from treatment temperatures below 180°C could be fermented (slowly) by ethanologenic *E. coli* without the need of purification [53]. This demonstrated low level of potential inhibitors.

In another study, hemicelluloses from sugarcane bagasse were efficiently solubilized (96% and 98% after 8 and 24 min, respectively) using a low concentration of phosphoric acid (0.20%) at 186°C [54]. Enzymatic cellulose conversion of pretreated bagasse using 20 filter paper cellulase units (FPU) $g^{-1}$ of Novozymes Celluclast® (a commercial cellulase preparation produced by a selected strain of the fungus *Trichoderma reesei*) treated under these conditions of pretreatment produced the highest cellulose conversion of 56.38%. In general low levels of degradation products were achieved; however, minor increase of these products were observed when temperature was elevated to 186°C that can be explained by the high solubilization of hemicellulose fraction at this condition [54]

Mild phosphoric pretreatment has been also adopted with stream treated substrates. Preimpregnation of *Eucalyptus benthamii* with diluted phosphoric acid followed by steam explosion resulted in an improved selectivity towards hemicellulose hydrolysis (xylose yields of 50–60%), yielding substrates readily susceptible to saccharification with Novozymes Cellic® CTec2 (a commercial enzymatic blend to produce cellulosic ethanol) at relatively high solids (10%) [55].

Results obtained on sugarcane bagasse through a central composite design comparing steam explosion carried out in the absence (autohydrolysis) and presence of phosphoric acid showed that phosphoric acid catalysis (19 mg $g^{-1}$) resulted in better glucan yields under milder conditions (180°C, 5 min) [56]. Phosphoric acid catalysis produced steam-treated substrates with good susceptibility to enzymatic hydrolysis (30 mg $g^{-1}$ Cellic® CTec2, at 8% of substrate consistency) yielding in average 75% of glucose.

Current wheat based bioethanol production (first generation) depends significantly on DDGS (distillers dried grains with solubles), a common byproduct that is sold separately as animal feed [57]. Phosphoric acid has been shown as a viable option to maintain substrate quality without contaminating the feed residues with high sulfur levels encountered if $H_2SO_4$ as used. In a recent study, dilute phosphoric acid pretreatment was optimized for wheat straw in laboratory scale and the results validated for the first time in a Biorefinery Demo Plant (BDP), operated by SP (Technical Research Institute of Sweden) at Örnsköldsvik, Sweden [58]. Optimal pretreatment conditions were determined in the laboratory as an acid concentration of 1.75% (w/v) at a temperature of 190°C for 15 min, based on the maximum enzymatic digestibility with the minimum inhibitor release. Enzymatic polysaccharide hydrolysis reached 36% for untreated straw and 86% for straw pretreated with dilute phosphoric acid. Based on this, scale up of the acid phosphoric pretreatment was applied at the biorefinery demonstration plant and an improved efficiency of polysaccharide hydrolysis was obtained (95% of the theoretical maximum). Further sugar fermentation by the Ascomycete *Neurospora intermedia* showed an improvement in the ethanol yield from 29% (with untreated straw) to 94% (with dilute phosphoric acid pretreated straw) of the theoretical maximum.

## 5. Starch hydrolysis with diluted phosphoric acid

Starch is the second most abundant polymer in the world [59]. Starch granules are biosynthesized reserve polysaccharide in a broad array of plant tissues and within many plant species. Potatoes and cassava are outstanding starch sources. They are composed of two types of $\alpha$-linked glucans: amylose, a straight chain of $\alpha$-1,4-linked glucopyranosyl units and amylopectin, which has besides $\alpha$-1,4-linked glucopyranosyl units various branch points with $\alpha$-1,6-linkages. A linear polymer of amylose (around 20% of whole starch) can have up to 6000 glucose units, whereas amylopectin (around 80% of the whole starch) is composed of $\alpha$-1,4-linked chains of 10–60 glucose units with $\alpha$-1,6-linked side chains of 15–45 glucose units. Both building blocks represent approximately 98–99% of the starch dry weight [60].

Starch may be chemically, enzymatically or physically modified to produce a broth rich in glucose that possess potential use in biotechnological processes, such as fermentation substrate for microorganisms to produce bioethanol, enzymes and other biomolecules. It can be also modified to present novel characteristics, creating innumerous applications, as for example in the food industry, as sweetener or thickening and gelling agent. Enzymatic conversion of starch to free glucose requires the concurrence of two enzymes: $\alpha$-amylase, that yields malto-oligosaccharides and dextrins of varying chain length, and $\alpha$-(1,4)-glucosidase (maltase), which hydrolyses terminal, non-reducing $\alpha$-1,4-linked D-glucose residues with release of free D-glucose. These two enzymes can be replaced by amyloglucosidase (glucoamylase), a single enzyme able to break simultaneously the $\alpha$-D-(1-4) and the $\alpha$-D-(1-6), glycosidic bonds of both poly- and oligosaccharides. Efficient amylase-producing species include those bacteria of genus *Bacillus* (e.g. *B. licheniformis, B. subtilis, B. stearothermophilus, B. amyloliquefaciens*)

and fungi of genus *Aspergillus* (e.g. *A. niger, A. oryzae, A. awamori, A. fumigatus*). Amylases makes up today up to 25% of the world enzyme market (personal communication, August, 2017), and are together with proteases, the most versatile enzymes in the industrial enzyme sector because of the abundance of substrates, raw materials and variety of applications as bakery goods, sugar products, biofuel industry, and many others.

Starch can be modified by chemical methods and an example are those termed "acid-thinned", normally used for food and beverage applications that involve an existing high starch content. Both $\alpha$-1,4 and $\alpha$-1,6 glucosidic linkages are moderately resistant to acid hydrolysis, with the amorphous regions of the granule more susceptible to chemical treatment than the crystalline regions. Acid modified starches are prepared industrially by treating the starch slurry (40%) with varying concentrations of mineral acids and hydrolysis time at temperatures below that of gelatinization (25–55°C) [61]. Acid treatment increases the gelatinization parameters (gelatinization temperature and enthalpy), reduces the molar mass and viscosity, increases the solubility of the granules, minimizes syneresis (separation of liquid from a gel caused by contraction), and causes gel thermo-reversibility when subjected to cooling after melting [61].

Acid Hydrochloric and sulfuric acid, more often the second, are the generally used mineral/inorganic acids for starch hydrolysis, but they can present several problems. When using hydrochloric acid, in downstream step is necessary to desalinate the syrup using high cost ion exchange resins. Additionally, undesirable byproducts are produced even when syrups of an average dextrose equivalents are produced, because free glucose is converted to dehydration products such as hydroxymethylfurfural (HMF), levulinic and formic acids, which in turn can inhibit microbial growth if a subsequent fermentation step is required [62]. Furthermore, these mineral acids can easily produce toxic gases in the course of the process [63]. In the food and beverage industry, for example, another problem arises from the Maillard reactions between reducing sugars and R-NH2 groups from amino acids and proteins. This negative occurrence is designed as "mud" in the starch-processing factories of glucose-enriched syrups. Its worse properties are brown color and bitter taste [63].

The use of phosphoric acid instead of the stronger mentioned acids presents several advantages: safer handing because is a non-volatile acid, reduced byproducts formation and if the hydrolysate is to be used in a subsequent fermentation step, there is no need to remove or eliminate the phosphoric acid catalyst. Instead, neutralization with ammonia leads the formation of ammonium phosphate, a convenient supplement for growth as P and N-source. Taken together, these various considerations create the assumption of phosphoric acid as the preferred acid catalyst. From a strict biochemical stand point, let us to recall how much phosphoric acid is a "body friend" molecule: it is present in DNA, ATP, casein and phosphoric-esters (Gluc- and Fruct-phosphates feeding the universal glycolytic pathway).

Our study comparing hydrolysis with phosphoric acid and hydrochloric acid on cassava starch paste (30%, w/v) has shown that at 160°C (*ca.* 6 atm), the desired dextrose equivalent (DE) was obtained with both acids: DE value of 85 at pH = 1.6 and pH = 1.8 using hydrochloric acid and a DE value of 83 at pH = 1.4 with phosphoric acid (**Figure 2**) [63]. Higher

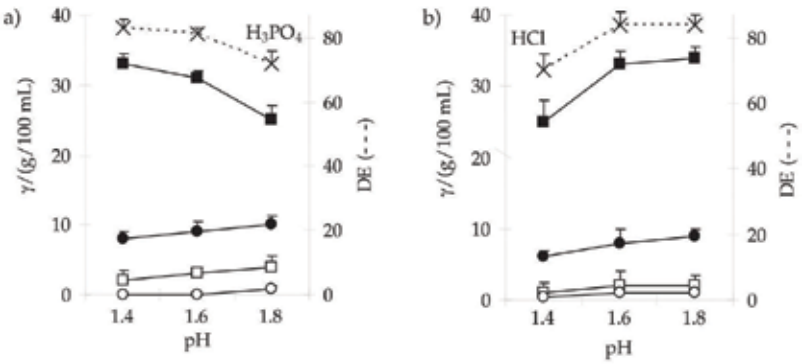


**Figure 2.** Effect of pH on the hydrolysis of cassava starch (30%) by phosphoric acid (a) and hydrochloric acid (b) at the temperature of 160°C (5 bar) during 10 min. Final concentrations of hydrolysis products and the final DE are shown. Key: (– x –) dextrose equivalents (DE), (■) glucose, (●) maltose, (□) maltotriose, (○) maltotetraose and higher.


**Figure 3.** Example of sweeteners and GRASE status (generally regarded as safe and effective) of phosphoric and/or citric acids or their salts. (A) Cola soft drink containing HFCS or steviol glycosides and phosphoric acid; (B) fermented milk containing sweeteners from corn (high-fructose corn syrup - HFCS and/or modified corn starch) phosphoric and/or citric acids or their salts (Source: Personal photo, 2015).

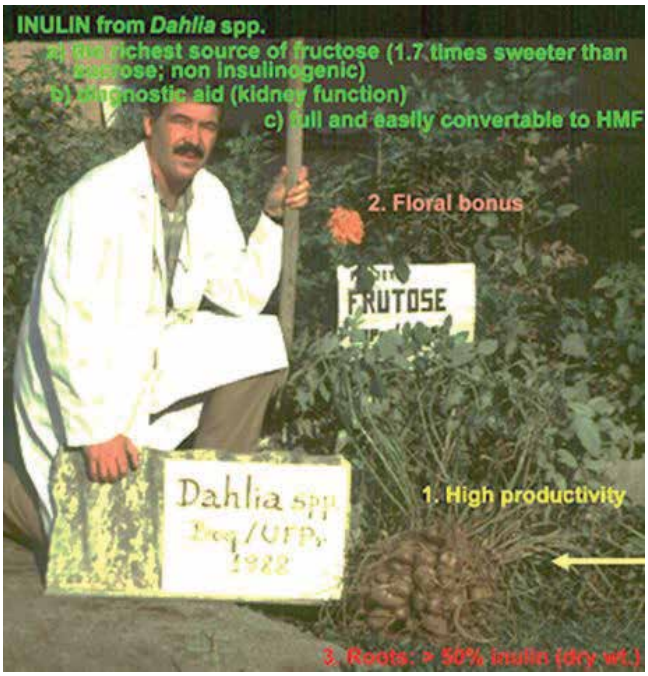


**Figure 4.** *Dahlia* sp. garden cultivation offers the most productive source for inulin which through a quick extraction of decorticated and sliced tubercles with pH 7 buffered hot water, followed by polymer retrogradation in a cold (ca. 8°C) environment. (Source: Prof. J. D. Fontana private auto-photo album).

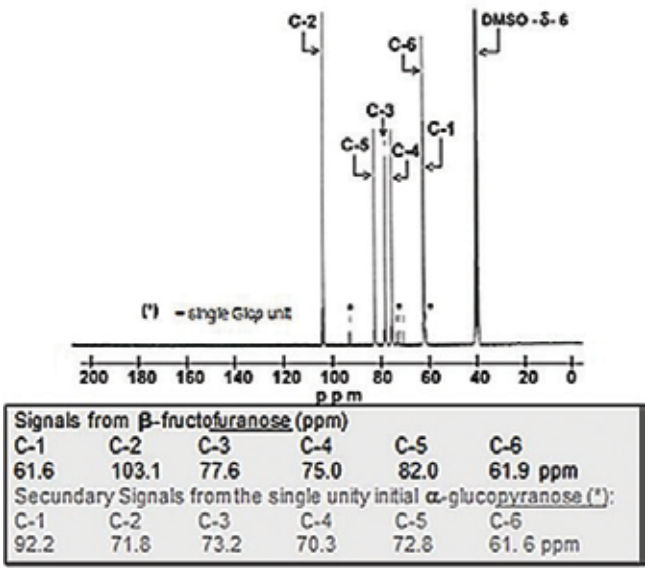


**Figure 5.** 13C-Nuclear Magnetic Resonance (NMR) of purified *Dahlia* sp. tubercles inulin. The presence of minor spectroscopy signals – asterisk labels – correspond to the single glucopyranosyl of each whole inulin molecule, thus revealing that extraction and purification steps were carefully carried and preserving the polysaccharide native chemical structure. (Source: authors lab associate).

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temperatures and lower pH values led to higher concentrations of HMF and formic acid with both acids, but these quantities were always lower when hydrolysis was carried out with phosphoric acid (e.g. for HMF at pH = 1.5 and 152°C, 4 bar with a holding time of 5 min: 0.185 mg/mL using HCl against 0.075 mg/mL with $H_3PO_4$) [63].

Our oPA-mediated cassava starch hydrolysate allowed biomass growth and astaxanthin production by the heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous* (formerly: *Phaffia rhodozyma*) with parallel consumption of all maltosugars from G2 to G6 from an initial 64% of reducing sugars) reaching a maximum 3.34 mg/L of astaxanthin in a culture medium containing 6.5% w/v starch hydrolysate with supplementation (0.05 g/L yeast extract, and 25 to 50 mg/L of $NH_4NO_3$ [63]. Results have shown that that diluted thermopressurized phosphoric acid can be used as alternative catalyst to produce high DE syrups from cassava and other starch sources, residual phosphate being left in the final hydrolysate (after a light neutralization with ammonia or other alkalis) as a fermentation co-nutrient feedstock to produce biomolecules [63].

It may be remembered that sweetening of soft drinks and fermented milk (lacteous beverages and yogurts) can be attained with several alternatives of natural sugars or artificial sweeteners. Any of them are always accompanied with some phosphoric or citric acid and/or their salts, as shown in the following illustration with two worldwide sold products (**Figure 3A** and **B**).

oPa-catalyzed partially starch hydrolysates (and even better if fructo-oligosaccharides, commonly known as FOS, the nutraceutical and anti-tumor oligosaccharides of inulin) along with the retained oPA catalyst within the hydrolysates may be a clever strategy to these product industrial formulas. Our group is waiting for the patent request PI 0703206-4 grant from the Brazilian INPI—National Institute for Industrial Property. Its claim includes the protection of the utilization of phosphoric and citric acids as mild catalysts for the production of FOS from *Dahlia* sp. tubers inulin. One example of such occurrence and some properties of inulin are shown in the **Figures 4–7**.

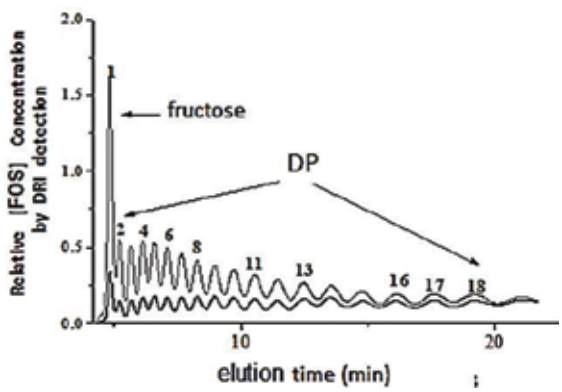


**Figure 6.** High performance liquid chromatography (HPLC) monitoring of the degree of polymerization (DP) of oPA-partially hydrolyzed 10% inulin with pH 2.0 at 85°C for 5 min (bottom line) or 15 min (upper line). (Source: Authors lab). FOS: fructo-oligosaccharides; RID: refractive index detector.

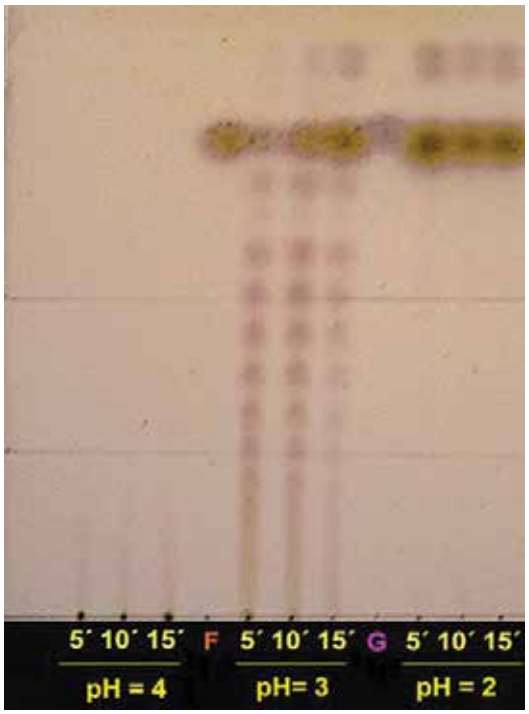


**Figure 7.** Thin-layer chromatography (TLC) monitoring of inulin (poly-D-fructofuranose) by oPA-catalyzed partial or total hydrolysis along 15 min of incubation at 75°C: pH 4 (*left*), pH 3 (*center*) and pH 2 (*right*). Revelator: Hot 0.5% orcinol in MeOH:$H_2SO_4$ 9:1. The major spot at Rf = 0.8 is free fructose (F´). The multiband profile (*right*) are FOS (Fructo-oligoSaccharides) with degree of polymerization (DP) from 2 till 10. The two spots ahead fructose are HMF (HydroxyMethylFurufuraldeyde) and probably some DFA (DiFructose anhydride) due to acid reversion of free fructose.

## 6. Nutraceutical oligosaccharides products obtained by diluted thermopressurized phosphoric acid treatment of microalgae cell walls

Our more recent application of the oPA-mediated catalysis of polymeric sugars has been recently published [64]. The hydrolysis substrates, initially, were microalgae cell walls. This is a very convenient approach to be coupled to microalgae whole biomass once extracted with hot organic solvents (e.g., anhydrous ethanol) to preliminary redeem the lipid material for biodiesel production. In fact, our prospection in these so intensively explored marine unicellular microorganism has gone far: the microalgae (*Chlorella vulgaris*) and cyanobacteria or blue-green algae (*Arthrospira platensis*; formerly *Spirulina platensis*) biomasses, coming from photobioreactors or large open bowls installed in the open roof of local steak house were permanently bubbled with the whole but filtered gases and other volatile components of the hot stream arising from the grills and driven to the bottom of photobioreactors and bowls with the help of a fan. Finalizing, the following chromatographic illustration – variable series of oligosaccharides – confirms the validity of this technology novelty (**Figure 8**).

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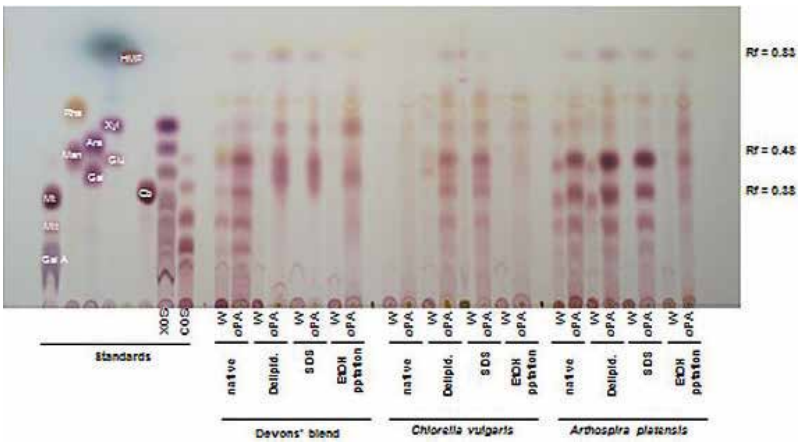


**Figure 8.** Thin-layer chromatography (TLC) of Nutraceutical oligosaccharides (NOs) arising from diluted thermopressurized oPA-catalyzed treatment of two microalgae and one cyanobacterium cell walls. Real effective pH 2.0 (after equilibration and complete wetting of each microorganism cell mass) and then a thermopressurization at 4.5 atm (156°C) till the peak condition for 2 minutes. Mobile phase: Acetonitrile:Isopropanol:Water (15:3:5). Chromogenic reagent: Orcinol in sulfuric acid. Standards: (Gal A) galacturonic acid, (Rha) rhamnose, (Man) mannose, (Ara) arabinose, (Gal) galactose, (Xyl) xylose, Rf = 0.48 - (Glu) glucose, Rf 0.38 - (Cb) cellobiose and (Mt) maltose, Rf = 0.29 - (Mtt) maltotriose, (XOS) xylo-oligosaccharides, (COS) cello-oligosaccharides, Rf = 0.83 - (HMF) hydroxymethylfurfural. (Source: Authors; picture abstracted from Bruna Leal master dissertation, supervised by Prof. Marcelo R. Prado and Adéia Grzybowski, 2015).

## 7. Conclusions

Great potential is observed in the deconstruction of phytobiomass polysaccharides to its component sugars. Resulting monomers and/or oligomers can be used for the production of a plethora of products, with application to biofuels, food, fine chemicals and other industries. As mentioned before, due to the inherent characteristics of phosphoric acid, it can be used as an advantageous catalyst for the depolymerization of polysaccharides from a great variety of phytobiomass. Pretreatment technology using very diluted phosphoric acid, alone and under moderated thermopressurization for the bioprocessing of a sugarcane and other L(h)C substrates can possess important advantages over the use of mineral/inorganic acids, despite its relatively higher cost when compared to sulfuric acid, for example. We have shown the potential for using phosphoric acid hydrolysates to fermentation processes using different microorganisms, to the production of bioethanol, to increase nutritional value in animal feed, for starch modification, biomass growth and in the production of prebiotic/alternative sweetener (fructo-oligosaccharides). A continuous study in the use diluted phosphoric acid on different biomass could improve strategies that can be further used in industry and biorefinery processes.

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Chapter 14

# Sugarcane Bagasse As Potentially Low-Cost Biosorbent

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Additional information is available at the end of the chapter

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**Abstract**

Sugarcane bagasse (SB) is one of the major residues obtained from agriculture, every year millions of tons of SB have been produced by the sugarcane agribusiness. This abundant residue has been showed potential as biosorbent in wastewater treatment. SB, *in nature* or chemically modified, has been widely reported as a promising sorbent for the removal of dyes or heavy metals from aqueous medium. The application of SB in oil sorption is rarer, especially for the treatment of used motor oil wastewater. However, in this chapter, we show that this material has good oil sorption capacity when compared to other commercial and natural sorbents. This study evaluates the effect of several coupling agents over SB in used motor oil sorption as well as the influence of surfactant in this process.

**Keywords:** engine washing wastewater, oil sorption, sugarcane bagasse, dye sorption, biomass

## 1. Introduction

Agricultural waste by-products, such as sugarcane bagasse (SB), rice husk, coconut husk, sisal, and so on, have been extensively studied as a potential sorbent material for removing contaminants from water and wastewater [1]. These materials have many advantages such as the abundance, low cost, floatability, good flexibility and mechanical strength, and environmentally friendly properties. Besides, the potential use of several agricultural by-products as biosorbent is supported by its native adsorption capacity derived from their main constituents such as cellulose, hemicellulose, and lignin. These are polymeric structures with high content of hydroxyl and carboxyl groups, which have a strong influence in the adsorption capacity of different chemicals present in the aqueous medium.

SB, in nature or chemically modified, has been reported as a potential renewable sorbent for wastewater treatment [2, 3]. Millions of tons of SB have been generated every year by the sugarcane agribusiness, encouraging its reuse and recycling. The sugarcane industry is based mainly on the production of sugar and ethanol, which generates huge volumes of SB and sugarcane trash [4, 5]. After sugarcane is milled for juice extraction, bagasse is obtained as a residue, which corresponds to about 25% of the total weight, and is composed of approximately 40% cellulose, 24% hemicellulose, and 25% lignin. The hydroxyl groups are the most abundant and reactive sites in this biopolymer and are used to attach a variety of functional groups [6]. The higher content of cellulose in the SB biomass favors its hydrophilicity, which improves its interaction with cationic species in aqueous medium. As a result, SB has been widely used for the removal of heavy metals and dyes from wastewater [7–9].

### 1.1. Sugarcane bagasse as a sorbent for heavy metals removal

The utilization of unmodified or modified SB as an adsorbent have been described as a cheaper and effective technology for the removal of metal ions from wastewater [10, 11]. The metal ion-binding mechanism of adsorption on SB is attributed to its abundance of hydroxyl groups from cellulose, in which aqueous medium favors ion exchange or complexation with metal ions. Batch studies [12] using natural SB as a sorbent for removal of Cd(II) show the maximum adsorption at pH 6. The pH dependence of Cd(II) uptake was linked to both the surface functional groups and the metal ion species predominant in aqueous solution. The species $Cd^{2+}$ and $Cd(OH)^{+}$ are predominant at pH lower than 6, while the groups on surface are protonated and cannot bind to metal ions in solution. Besides, at very low pH, the surface groups are associated with the hydronium ions ($H_3O^{+}$), negatively affecting the interaction with the metal cations. When the pH increases, the surface affinity with the metal also increases, and adsorption is improved.

The metal ion-binding capacity of SB can be intensified by the introduction of surface groups with capacity chelating as carboxylate or amine [13–15]. The introduction of carboxylic functions (−COOH) on cellulosic fiber, lignin, and hemicellulose can be performed via cyclic anhydride reaction. **Figure 1** displays an example of succinylation [16] reaction as an alternative route to attach carboxylic acid onto the cellulose.

Pereira et al. describe the chemical modification of SB by ethylenediaminetetraacetic (EDTA) dianhydride (EDTAD) in order to improve $Zn^{2+}$ adsorption [14]. The EDTA molecule is a

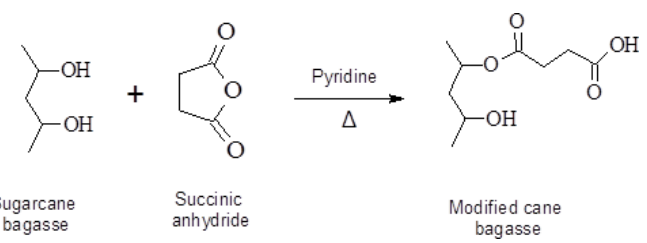


**Figure 1.** Scheme of possible synthetic route for introduction of carboxylic groups.

chelating group that enhanced the metal complexation on fiber surface. Other compounds such as citric acid and phthalic anhydrides have also been used for SB modification, resulting then in an increase of adsorption capacity for these fibers [9, 13, 17, 18]. **Table 1** summarizes some studies where SB in nature or modified form is used to remove heavy metals from aqueous environment.

### 1.2. Sugarcane bagasse as an adsorbent for removal of dyes

With the expansion of textile sector, dyes in wastewater have become a serious environmental problem. Dyes are organic chemical compounds that appear colored due to the presence of chromophore groups such as nitrous, azo, and carbonyl [19, 20]. The release of dye waste into water bodies affects the life in aquatic environments, causing the ruining of soils and poisoning

| Metal ion | SB/modification | Sorption capacity (mg $g^{-1}$) | References |
|---|---|---|---|
| $Cd^{2+}$ | SB without modification | 69.06 | [12] |
| $Zn^{2+}$ | Ethylenediaminetetraacetic dianhydride | 105.26 | [14] |
| | | 45.45 | |
| $Pb^{2+}$ | Without modification | 26.67 | [17] |
| | *Pleurotus ostreatus* (U2–11) | 36.31 | |
| | *Lentinula edodes* (U6–1), | 27.68 | |
| | Basidiomycetes | 39.93 | |
| | *Ganoderma lucidum* (U12–6) | 36.00 | |
| $Co^{2+}$ | Trimellitic anhydride | 1.153 | [3] |
| $Cu^{2+}$ | | 0.979 | |
| $Ni^{2+}$ | | 0.849 | |
| $Cu^{2+}$ | Tetraethylenepentamine | 0.016 | [18] |
| $Cu^{2+}$ | Succinic anhydride | 114 | [15] |
| $Cd^{2+}$ | | 196 | |
| $Pb^{2+}$ | | 189 | |
| $Co^{2+}$ | Phthalic anhydride | 0.561 | [3] |
| $Cu^{2+}$ | | 0.935 | |
| $Ni^{2+}$ | | 0.932 | |
| $Cr^{3+}$ | Without modification | 16.21 | [13] |
| | NaOH | 26.41 | |
| | Citric acid | 17.2 | |
| | NaOH/acylation with citric acid | 31.28 | |

**Table 1.** SB in nature or modified as metal sorbent reported in the studies.

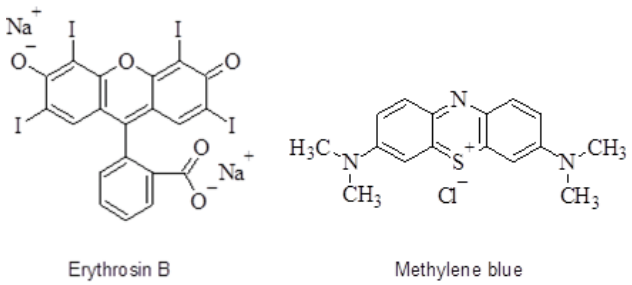


**Figure 2.** Example of cationic (erythrosin B) and anionic (methylene blue) dyes.

of drinking water. Besides, dyes cannot be removed by conventional treatment methods, and are resistant to aerobic digestion. As an alternative method, physical removal of dyes from effluent through biosorption has been extensively studied [8, 21]. A dye molecule is characterized by the presence of chromophore groups, which are responsible for producing the color, and also by groups known as auxochromes such as carboxylic acid, sulfonic acid, amino, and hydroxyl groups. These auxochromes are responsible for impacting or shifting of a particular color when attached to a chromophore, and also used to influence the dye solubility. In fact,

| Dyes | SB/modification | Sorption capacity (mg $g^{-1}$) | References |
|---|---|---|---|
| Crystal violet | Oxidation with $H_3PO_4$-$NaNO_2$ | 1018.2 | [8] |
| Auramine O | | 571.8 | |
| Methylene blue | Maleic anhydride | 30.4 | [25] |
| Rhodamine B | Without modification | 51.3 | [2] |
| Methylene blue | | 28.0 | |
| Acid Alizarin Violet N | Without modification | 20.8 | [26] |
| Methyl red | Phosphoric acid | 11.0 | [21] |
| | Without modification | 5.7 | |
| Congo red | Without modification | 39.8 | [24] |
| Methylene blue | Ethylenediaminetetraacetic dianhydride | 115.3 | [27] |
| Crystal violet | Meldrum's acid | 552.7 | [7] |
| Methylene blue | Ethylenediaminetetraacetic dianhydride | 192.3 | [19] |
| Gentian violet | | 357.1 | |
| Methylene blue | Succinic anhydride | 434.3 | [16] |
| Gentian violet | | 1133.7 | |
| Erythrosin B | Without modification | 333.3 | [22] |
| Methylene blue | | 1000.0 | |

**Table 2.** Examples of dyes adsorption by SB.

the auxochromes are important to enhance the affinity of the dye toward the fibers. As a result, natural fiber, as SB, presents greater potential to remove dyes from wastewater.

Basically, dyes can be classified as cationic or anionic. **Figure 2** shows, for example, the molecules of erythrosin B (EB) and methylene blue (MB). Cationic dyes carry a positive charge in their molecule, while anionic dyes carry a negative charge [22–24]. In aqueous solution, the dye molecules will present positively or negatively charged as a function of pH, and the electrostatic interaction with the fiber surface will direct the adsorption process. Therefore, the dyes adsorption route by SB can be described in a similar way as observed for metal ions. At low pH, the surface of SB became positively charged and the cationic dye adsorption will decrease, while for anionic dyes the reverse process occurs. In contrast, at high pH, the cationic dye removal will increase because the surface appears negatively charged and the anionic dye adsorption became inhibited. **Table 2** summarizes some results of adsorption of dyes using SB, natural or modified, as a sorbent.

## 2. Sugarcane bagasse-based sorbents for motor oil removal

Research involving oil sorbents was firstly encouraged by the great environmental accidents generated by oil spills at sea [28–30]. In these cases, the adsorption processes are more suitable to remove and recover the oil. The sorbent material facilitates a transformation from liquid to solid phase, and then oil can be removed together with the sorbent. The main characteristic of crude oil sorbent material is the hydrophobicity and oleophilicity in order to attract oil preferentially to water. However, the amount of sorbents added to an oil polluted environment is a critical factor, because the inappropriate and excessive use can present subsequent waste disposal problems. It is especially important when organic synthetic products are used as sorbents. Synthetic sorbents, as polypropylene, do not degrade and are very expensive. Therefore, agricultural waste by-products were firstly used as an alternative oil sorbent to replace the conventional and non-degradable sorbent used to clean up oil spills. These biosorbents are biodegradable, renewable, abundant, and low cost. Teas et al. [31] compared the oil sorption capacity of cellulose with the expanded perlite and polypropylene in artificial seawater containing crude oil. These authors observed that for crude oil, the sorption capacity of cellulose overtakes the other sorbents. When light cycle oil and light gas oil were used in artificial seawater, they observed lower sorption by cellulose in relation to polypropylene, but similar behavior to expand perlite. The oil sorption capacity of vegetal fibers observed by several authors has been attributed to the interaction with hydrophobic sites in the biomass. Lignocellulosic fibers contain both hydrophilic and hydrophobic groups; however, the cellulose structure has hydrophilic nature with excellent wettability. The chemical functionalization of cellulose can increase its hydrophobic character, which is possible by changing the hydrophilic groups, hydroxyl (−OH) in the raw coir cellulose to hydrophobic hydrocarbons [32–34]. The biomass acetylation has been extensively used to increase its oil sorption capacity. Sun et al. [35] observed that SB acetylated presents greater machine oil sorption capacity (13.5–20.2 g $g^{-}1$) than polypropylene fibers (10 g $g^{-1}$). **Table 3** summarizes other studies that have been demonstrated the potential sorbent of agricultural by-products to oil removal and the effect of biomass modification in the sorption capacity.

| Adsorption/sorption conditions | Sorbent/treatment | | Sorption capacity (g $g^{-1}$) | References |
|---|---|---|---|---|
| Crude oil in dry conditions | *Dacryodes edulis* leaf | Unmodified leaf | 3.440 | [33] |
| | | Acetylated with acetic anhydride | 4.990 | |
| | Raw cotton | | 30.5 | [36] |
| Crude oil in distilled water | Sisal (*Agave sisalana*) | | 3.0–6.4 | [28] |
| | Coir fiber (*Cocos nucifera*) | | 1.8–5.4 | |
| | Sponge-gourd (*Luffa cylindrica*) | | 1.9–4.6 | |
| Crude oil in artificial seawater | Mangrove barks | Untreated bark | 1.666 | [37] |
| | | Treated with oleic acid | 3.333 | |
| | | Treated with palmitic acid | 3.333 | |
| Machine oil in distilled water | Sugarcane bagasse | Untreated bagasse | 8.9 | [35] |
| | | Acetylated with acetic anhydride | 11.4–16.5 | |
| | Sugarcane bagasse modified with acetic anhydride | | 13.5–20.2 | [38] |
| Synthetic effluent of crude petroleum | Sugarcane bagasse | | 6.65 | [39] |
| Motor oil in water | Natural wood fibers | | 33–43 | [40] |
| Wastewater of used motor oil | Natural wood fibers | | 5.56 | [41] |
| Engine oil in dry conditions | *Ceiba pentandra* (L.) Gaertn. fibers (Kapok) packing density 0.02 g $mL^{-1}$) | | 47.4 | [42] |
| Used engine oil in dry conditions | | | 50.8 | |

**Table 3.** Examples of oil sorption by biomass.

In spite of crude oil sorption favored by hydrophobic surface, in the case of used motor oil, this behavior is not completely precise. The crude oil and fresh motor oil composition is based on a mixture of hydrocarbons, and hence present affinity for hydrophobic sorbent. However, the composition of used motor oil has been changed through thermal degradation and contamination from generated waste in the engine. This process leads to the formation of low-molecular weight compounds and oxidation products [43]. Recently, Guilharduci et al. [44] showed that introduction of hydrophilic groups on SB improve the oil sorption from engine washing wastewater. Besides, it was also verified that surfactant present in wastewater affect the motor oil sorption.

The biomass acylation is well established in the literature, and the method is based on the reaction of hydroxyl groups (−OH) of the fiber surface with acyl groups (RCO–). Considering that the reaction with fiber is a heterogeneous reaction, not all hydroxyl groups is esterified,

and a nonuniform product may be obtained [32, 35, 38, 45]. In the case of SB, the acetylation with acetic anhydride should introduce the methyl group and increase hydrophobicity at surface. Other acylating agents such as maleic [46], phthalic [9], and succinic [16] anhydrides have been used to introduce carboxylic groups on fibers and increase its surface hydrophilicity.

Another class of coupling agents recognized as efficient to promote the fiber hydrophobicity or hydrophilicity are the silanes. They have been extensively used to modify the surface of natural fibers to produce composites with thermoplastics or thermosets [47]. The aminosilanes, as the aminopropyltriethoxysilane (APS), are the most reported coupling agents for natural fibers. Recently, APS was successful used to modify the SB surface in order to improve its hydrophilic properties, and increase its oils sorption capacity [44].

Despite the great demand for treatment of effluents containing used oil, there is a scarcity of literature on this problem. In this study, this subject is explored in order to verify the potential of SB fibers for oil wastewater treatment. The effect of several coupling agents over SB capacity for sorption of used motor oil is investigated as well as the influence of surfactant in this process.

## 2.1. Methodology

### *2.1.1. Reagents*

Acetic anhydride (99%), maleic anhydride (96%), phthalic anhydride (99%), succinic anhydride (97%), and aminopropyltriethoxysilane (APS) (97%) were purchased from Sigma-Aldrich and used without further purification. The other analytical grade reagents were obtained from Merck (Brazil) and were used as received. The used motor oil used in this study was obtained from Retifica del Rei (São João del Rei, MG, Brazil). SB was supplied by Cachaça Coqueiro (Nazareno, MG, Brazil). The bagasse was washed repeatedly with distilled water to remove all the dirt particles. The washed fiber was then dried in an oven (Q314M, Quimis) at 60°C for 24 h under a flow of air. It was subsequently ground and sieved through 30 mesh sieves (TE648, Tecnal). The resulting natural fiber was designated as SBN and was used as the starting material to produce the modified SB.

### *2.1.2. Fiber modification*

Thermal treatment of SBN at 200°C for 24 h was performed to obtain the sample SB-200. The SBN acylation was obtained based on previous reports [44]. Briefly, the fiber was firstly soaked in 10% NaOH solution (using a ratio of 1 g/1 mL) at 0°C for 2 h. The bagasse was then washed with Milli-Q water until neutral pH and dried at 60°C for 24 h. Following this procedure, 70 g of the cleaned bagasse was placed in a 1 L round-bottom flask containing 300 mL of acylating agents and 200 mL of acetic acid. The mixture was acidified by adding 1 mL of $H_2SO_4$ and maintained under agitation for 24 h at 60°C. At the end of this period, the solution was filtered and the product was rinsed first with ethanol and then with water until the pH reached around pH 7.0. After this procedure, the sample was dried at 60°C for

24 h. The acylating agents used were acetic anhydride, maleic anhydride, phthalic anhydride, and succinic anhydride, which produce the samples SB-Acet, SB-M, SB-P, and SB-S, respectively.

The procedure of SB silanization was based on previous report [44]. Basically, the SB is firstly washed with Milli-Q water at 60°C until no color was observed in the washed water, next the fibers are mixed with APS, using a 5/2 mass ratio of SBN/APS, and dispersed into 400 mL of acetone. The suspension was placed in a bottle with glass spheres and left on a roller-conveyor (TE500/1, Tecnal) for 24 h at 200 rpm. The excess of reagents were Soxhlet-extracted with acetone for 24 h. Subsequently, the fiber was dried in an oven at 60°C for 24 h, under a flow of air. The result sample was denominated as SB-APS.

*2.1.3. Fibers characterization*

Fourier transform infrared (FTIR) spectra were obtained in the range 400–4000 $cm^{-1}$, using a Perkin-Elmer 1720 FTIR spectrometer. The samples were mixed with KBr (Merck, spectroscopy grade) in an approximate ratio of 10/1. The resulting mixture was pressed into pellets and the spectra were acquired using 300 scans with resolution of 4 $cm^{-1}$. Determination of C and N contents was carried out with a Thermo Fisher Scientific, Flash 2000 Analyzer.

*2.1.4. Sorption experiments*

Sorption test was performed using a synthetic used motor oil/water mixture with an oil concentration of 10.0 g $L^{-1}$. The used motor oil sorption was determined by immersing 1.0 g of fiber in 100 mL of the solution of oil/water mixture. After 24 h, the sorbent was removed, and dried for 24 h at room temperature. The oil sorption was calculated using the following equation:

$$Q = \frac{m_f - m_i}{m_i} \quad (1)$$

where Q is the oil sorption capacity (g/g), $m_f$ is the total mass of dry sorbent (g) after sorption, and $m_i$ is the mass of dry sorbent before sorption (g). The water sorption was determined by the same procedure but using only water.

The experiments of oil sorption were also carried out in solutions containing sodium dodecylbenzenesulfonate in a proportion of 0.05–0.30% in the dispersion of used motor oil/water. The surfactant concentration was determined following the methodology established by the American Public Health Association (Water Environment Federation & APHA 2005).

**2.2. Results and discussion**

**Table 4** summarizes the samples prepared in this study. Results from elemental analysis of nitrogen and carbon in each sample are also presented in **Table 4**. It is noticed that the highest content of nitrogen obtained for SB-APS in relation to SBN fiber, which is attributed to amino groups from APS.

| Sample | Treatment | Elemental analysis | |
|---|---|---|---|
| | | N% | C% |
| SBN | *In nature* | 0.18 | 45.7 |
| SB-200 | 24 h at 200°C | 0.28 | 48.2 |
| SB-Acet | Modified with acetic anhydride | *Not detected* | 45.1 |
| SB-M | Modified with maleic anhydride | 0.12 | 44.8 |
| SB-P | Modified with phthalic anhydride | 0.14 | 46.1 |
| SB-S | Modified with succinic anhydride | *Not detected* | 43.1 |
| SB-APS | Modified with aminopropyltriethoxysilane | 2.15 | 42.5 |

**Table 4.** SB-based sorbents produced in this study.

The modification introduced on the SBN was evaluated by FTIR and results are shown in **Figures 3** and **4**. For SBN spectra, four peaks are clearly observed between 3600 and 3307 $cm^{-1}$ characteristic of stretching vibration of hydroxyl groups, which are associated with lignocellulose structure. Upon acylation with acetic anhydride, the intensity of ─O─H absorption band decrease because of hydroxyl groups reduction after reaction. In the same region of spectra, for SB-M only a diffuse band, centered at 3387 $cm^{-1}$, is observed, and can be associated to hydrogen bonding in the hydroxyl groups. Similar profile is observed for SB-P and SB-S, in which, it is possible to identify two peaks less intense, at around 3600 and 3186 $cm^{-1}$, also attributed to hydrogen bonding in the hydroxyl groups. These results confirm the modification of surface, which decrease the free hydroxyl groups at fiber surface. Successful esterification can be supported by three important absorption band around 1736, 1367, and 1242 $cm^{-1}$, correspondent to the stretching vibration of carbonyl groups (C═O), C─H stretching, and C─O stretching characteristic of ester molecules. These bands can be clearly observed for SB-Acet, SB-S, and SB-M, however, with less intensity for SB-P, which can be associated with possible lower acylation obtained for this sample.

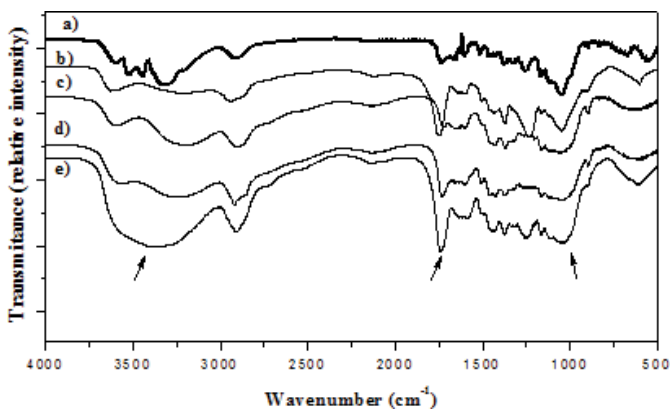


**Figure 3.** FTIR spectra of (a) SBN, (b) SB-Acet (c) SB-P, (d) SB-S, and (e) SB-M.

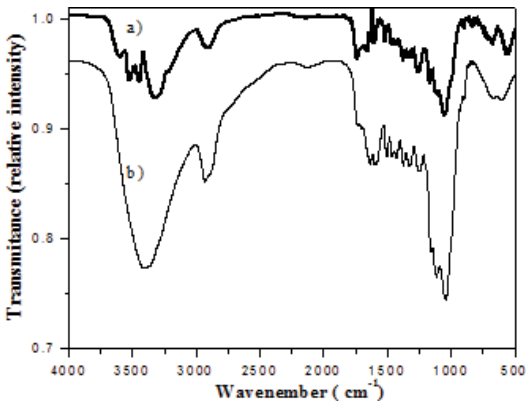


**Figure 4.** FTIR spectra of (a) SBN and (b) SB-APS.

For sample SB-APS, the FTIR is very similar to SBN (**Figure 4**). The characteristics of absorption bands of —Si—O—Si— at 700, 1030, 1145, and 1187 $cm^{-1}$ overlapping with groups present in the biomass surface hinder its identification [47]. However, in the region of 3600–3000 $cm^{-1}$, the APS modification leads to emerging of strong band, centered at 3398 $cm^{-1}$, attributed to hydrogen bonding from hydroxyl groups. This behavior suggesting that hydrophilic groups ($—NH_2$) from APS could be interacting with free hydroxyl groups.

The effect of SB modifications over oil and water sorption capacity can be observed in **Figure 5**. It is possibly observed that samples SB-APS, SBN, and SB-200 show higher affinity with water and also with used motor oil. This result suggests that the introduction of hydrophilic groups on SBN improve the used motor oil sorption. Guilharduci et al. [44] showed that used motor oil presents more affinity to hydrophilic surface than for crude oil. As previously discussed in the introduction, this behavior can be attributed to the chemical differences between used motor oil and new motor oil. Used motor oil can contain sludge, metal residues, and various

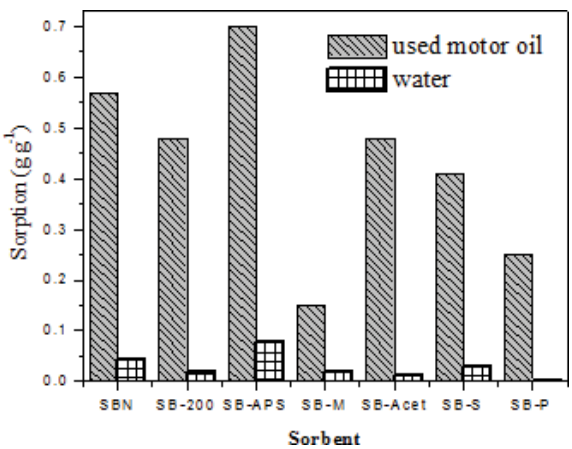


**Figure 5.** Oil and water sorption capacity for SB samples.

other contaminants. Therefore, the presence of polar amino end groups ($NH_2$) from silane structure favors the interaction with the constituents of the used motor oil. In contrast, the acylation of SBN was not effective to improve affinity with the constituents of used motor oil resulting in decreased adsorption capacity.

In spite of the higher oil sorption obtained for SB-APS (0.71 g $g^{-1}$) in relation to SBN (0.60 g $g^{-1}$), the difference was about 15%, which is relatively small. It is important to take in account that the fiber modification with silane groups is a costly and complex process. Considering the abundance, low cost and efficiency of SBN, this material presented the best cost benefit for use as sorbent of wastewater of used motor oil.

Previous studies showed that the surfactants in used motor oil wastewater can affect its sorption by natural fibers [44]. Surfactants are widely used in various industrial processes for oil recovery, because of their physicochemical characteristics for emulsification, dispersion, and solubilization [48]. They have the ability to reduce the oil-water interfacial tension, increase the capillarity, and change the wettability of the adsorbent surface. Then, in order to evaluate the impact of surfactants on the SBN sorption capacity, batch experiments were carried out using an anionic surfactant, sodium dodecylbenzenesulfonate, in a dispersion of oil (1.0 g)/water (0.1 L). The results are summarized in **Figure 6**(**a**, **b**).

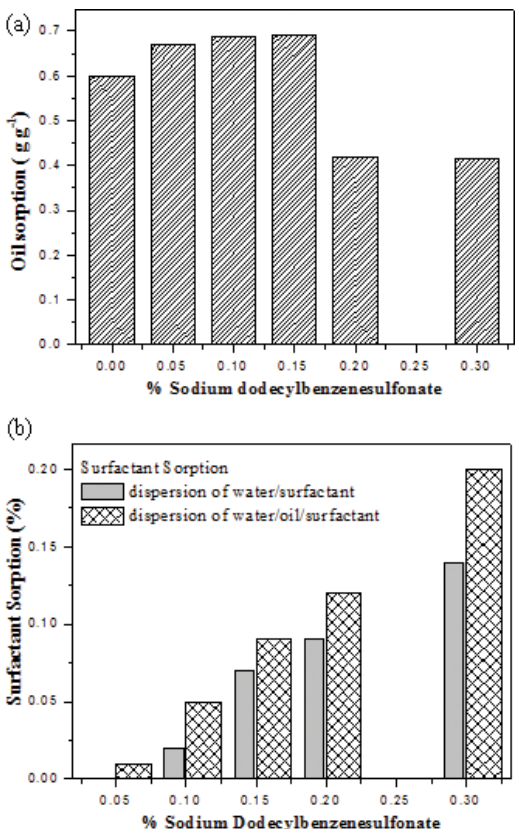


**Figure 6.** (a) The effect of anionic surfactant in the oil sorption and (b) surfactant sorption in dispersion with and without oil.

The used motor oil sorption shows slight increase (13%) in low concentration of surfactant, and afterward, the sorption decreases almost 42%. When the surfactant concentration increases, the hydrophobicity of fiber in aqueous solution decreased as a result of lower interfacial energy between water and fibers. In parallel, the oil-water interface is improved although micelles or microemulsions formation, which probably affects the oil sorption by SBN and improve surfactant interaction with the fiber. In this condition, the surfactant sorption is likely increased by SBN surface. This suggestion can be evaluated by the analyses of surfactant sorption in the water dispersion with and without oil. The results of these experiments are depicted in **Figure 6(b)**. Based on this result, it is noticed that surfactant sorption not only increases when its concentration increases but also is enhanced in the oil/water dispersion.

## 3. Conclusion

In this chapter, the great potential of SB in the natural and chemically modified form is shown to be applied as sorbent material for oil removal from aqueous medium. The performance of SBN to remove used motor oil from wastewater was around 0.6 g $g^{-1}$. This value increases around 13% in the presence of surfactant at 0.05–0.15% in water/oil dispersion. SB also showed efficient sorption of anionic surfactant in water dispersion with or without oil. The results support the use of SB as a natural sorbent as a substitute for commercial synthetic oil sorbents in the treatment of wastewater of used motor oil.

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Sugarcane (*Saccharum officinarum* L.) is considered one of the major bioenergy crops grown globally. Thus, sugarcane research to improve sustainable production worldwide is a vital task of the scientific community, to address the increasing demands and needs for their products, especially biofuels. In this context, this book covers the most recent research areas related to sugarcane production and its applications. It is composed of 14 chapters, divided into 5 sections that highlight fundamental insights into the current research and technology on this crop. *Sugarcane: Technology and Research* intends to provide the reader with a comprehensive overview in technology, production, and applied and basic research of this bioenergy species, approaching the latest developments on varied topics related to this crop.

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